METHODS OF ANALYSIS
A.O.A.C

OFFICIAL AND TENTATIVE

METHODS OF ANALYSIS

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS
W. W. Skinner (Chairman), J. A. LeClerc, L. E. Warren
J. W. Sale, G. G. Frary, and Marian F. Lapp

THIRD EDITION 1930

PUBLISHED BY THE
ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS
AT WASHINGTON, D. C.

PREFACE TO THIRD EDITION

The third issue of *Methods of Analysis* or *Book of Methods*, which are the abbreviated names of this publication, is offered to the members of the Association of Official Agricultural Chemists and to the public with a confidence that it will be as favorably received as were the previous editions That this book of methods as originally conceived and executed fulfills the aims of its sponsors and meets the needs of a large group of official and control chemists is evidenced by the continued and unexpected demand which exhausted—six months before this edition was ready for distribution—the second edition of five thousand copies The functions of the book and the philosophy of the development of the methods have been stated in the prefaces to former editions These prefaces are reprinted here for reference purposes

Although the arrangement is similar to that of the first and second editions, the changes, the rearrangements of data, and the additions and deletions are worthy of mention The subject matter is broadly grouped into two divisions, non-foods and foods The first division includes chapters on Soils, Fertilizers, Liming Materials, Insecticides, etc , thus bringing together related subjects, while the later chapters, devoted to foods, are arranged in alphabetical order, e g , Baking Powders, Beverages, Beers, Coffee, Cereal Products, etc This permits of easy reference and better meets the needs of a laboratory handbook Several independent chapters in former editions have been combined with chapters on related subjects, for example, the chapter on Vinegars has been combined with that on Spices and Other Condiments and the chapter on Gelatine has been included under Meat and Meat Products Beers, Wines and Distilled Liquors have been combined in one chapter The title Feeding Stuffs has been changed to Grain and Stock Feeds

An evidence of the progressive development of the work of the A O A C is the inclusion of new chapters on Caustic Poisons, Naval Stores, Paints, Radioactivity, and Eggs and Egg Products Chapter headings have been assigned to such subjects as Sewage, Fibers, Paper and Paper Materials, Nuts and Nut Products, etc , indicating new lines of work contemplated by the association Most notable among these new subjects are Vitamines, Microchemical Methods, and Bacteriological Methods The inclusion of these chapter headings, especially the last-named, may seem out of place, but the interests of the agricultural chemist have so broadened in recent years that he finds it necessary to be professionally equipped

to deal effectively with all those matters which come within the purview of research and control chemists Therefore it has seemed wise to provide an arrangement to meet future needs Indeed, the concepts of the profession of chemistry, especially as they apply to activities of the official chemist, have of necessity been extended to include physics, microbiology, bacteriology, microscopy, engineering, public health, etc

An innovation in this edition is the placing of chapter numbers on each side of the page at the top and the page number at the bottom This arrangement has facilitated editing by making it possible to include all cross references in the manuscript

As heretofore, the methods are classified as official and tentative In addition, note is made of those tentative methods which have received first action as official Second favorable action by the association is necessary, however, before these methods are finally adopted as official This classification is important to those who use these methods to support action before the courts, since they are accredited by the Secretary of Agriculture in law enforcement work and are also accepted by the States in regulatory activities In this connection it should be understood that the methods given under each caption apply in general only to the materials mentioned therein

That the third edition might not be unduly large owing to the volume of added material, and to make it possible to sell the book at the former price, the editors have resorted to abbreviations and contractions, and to certain forms of simplified spelling, as for example, cc for cubic centimeter, g for gram, soln for solution, temp for temperature, m p for melting point, c p for chemically pure, and the elements and common chemicals are expressed by symbols or formulas, as Cl, Br, Zn, HCl, $CaCl_2$, $KMnO_4$, etc In referring to the common acids and to ammonia the words "strong" and "concentrated" have been eliminated, it being understood that unless otherwise noted these reagents are the full strength product The letter "C" after degrees of temperature has been omitted, because unless otherwise stated all temperatures in this volume are expressed in degrees centigrade All tables have been rechecked, new ones inserted, and many of the old tables have been reduced in size when by so doing their usefulness was not impaired These changes have saved space equivalent to many pages of printed matter

While logically no part of *Methods of Analysis*, the editors, by a vote of the association, have included as an appendix the Definitions for Fertilizers

The organization for the work of the third edition differed materially from that of the first and second editions Upon the recommendation of R L Doolittle, Chairman of the Committee on Revision for both the first and second editions, the Executive Committee ordered the preparation

and printing in *The Journal* and in separates of all changes that had been made in the methods in any one year The purpose of this plan was to facilitate the compilation of these changes at the time of revision

The Editorial Committee appointed by the association for the third edition consists of W W Skinner, Chairman, J A LeClerc, J W Sale, L E Warren, G G Frary and Marian E Lapp The cheerful and generous help given to the committee by the various general referees, associate referees and former referees has made this revision possible Among those who deserve special mention are the following S Alfend, C H Badger, E M Bailey, L H Bailey, R T Balch, G L Bidwell V B Bonney, I D Clarke, P A Clifford, M R Coe, J Davidson, O L Evenson, G S Fraps, W C Geagley, J J T Graham, V E Grotlisch, B G Hartmann, A M Henry J T Keister, R H Kerr, J C Krantz, Jr C F Jablonski, G S Jamieson, C S Ladd, H A Lepper, W V Lander, H C Lythgoe, W H MacIntire, J S McHargue, R M Mehurin, A R Merz, V E Munsey, E M Nelson, A E Paul, W H Ross, G C Spencer, F P Veitch, H J Wichmann, J B Wilson and O B Winter

The work of the committee was organized by the selection of J A LeClerc as administrative secretary and Marian F Lapp as assistant To the painstaking and diligent efforts of these two members of the Revision Committee special credit for the compilation and prompt issue of the 1930 edition is due

W W Skinner
Secretary, Association of Official Agricultural Chemists, and Chairman, Board of Editors

Washington, D C
December 31, 1930

PREFACE TO SECOND EDITION

The methods of the Association of Official Agricultural Chemists are unique in several respects They are the outgrowth of continual critical collaborative trial or test participated in by a large number of workers and undertaken in order to establish the accuracy of analytical results They are subjected to a scrutiny of phraseology to insure clarity, probably unequaled in developing any similar methods They are formulated solely by responsible Federal and State officials acting together and thus are based on underlying principles of equity The association has always en-

couraged to the utmost the cooperation of representatives of interested industries, but it has jealously reserved the final formulation of its methods to official chemists Consequently, these methods have attained an enviable position in the fields of activity occupying the attention of the association and are accepted as authoritative in matters at issue before the courts, both Federal and State

As a result of the collaborative investigations conducted under the referee system, deletions, additions, and revisions in the methods are constantly being made From 1884 to 1894 the methods of analysis adopted by the association were published each year, with the secretary's report of the proceedings of the annual meeting, as a bulletin of the Division of Chemistry of the United States Department of Agriculture In 1895 the methods, brought up to date to include the changes sanctioned by the 1895 meeting, were printed as Division of Chemistry Bulletin 46, which was later revised to incorporate the changes subsequently made at the annual meetings up to 1899 The provisional methods for the analysis of foods, authorized by the 1901 meeting, were issued in 1902 as Bureau of Chemistry Bulletin 65 In 1907 the official and provisional methods as adopted by the association up to that time were printed as Bureau of Chemistry Bulletin 107, which was revised in 1908 From 1903 until 1912 circulars giving the official changes in the methods were issued annually, soon after each meeting In 1920 the association published the methods of analysis, revised to November 1, 1919, in book form under the title "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists"

This revision, authorized in 1922, contains all the changes in methods adopted at the 1919, 1920, 1921, 1922, and 1923 meetings, as well as the polarimetric methods for the determination of sucrose and the methods for the determination of moisture and ash in wheat flour adopted in 1924

The general plan of the original book has been retained The cross-references, however, are *to page and section, not to chapter and section,* and the following changes in the text have been made Two new chapters, one on agricultural liming materials and one on gelatin, have been added, the chapter on water has been expanded to include brines and salts, the methods for sugar products formerly included in the chapters, Foods and Feeding Stuffs and Saccharine Products, have been combined in a chapter entitled Sugars and Sugar Products, and the methods for stock feeds have been rearranged under the chapter heading Feeding Stuffs

The Committee on Editing Methods of Analysis of the association, consisting of R E Doolittle (chairman), G W Hoover, W H MacIntire, A J Patten, B B Ross, and J W Sale, prepared this revision Miss Marian E Lapp, associate editor of *The Journal* gave the committee valuable assistance and edited the manuscript Referees, associate referees,

and other members of the association assisted in compiling and critically reviewing the work

W W SKINNER
Secretary, Association of Official Agricultural Chemists

January 1, 1925

INTRODUCTION TO SECOND EDITION

In the present publication, the technic of agricultural analysis is brought up to date The most valuable contribution made to agriculture in the last forty years has been that of the standardization of the chemical and physical methods of research in agriculture by this association The importance of sound and accurate laboratory methods is not so highly appreciated as it should be Such methods are of supreme value not only to the agricultural worker, but also to all research workers in every branch of science, and especially in chemistry A striking illustration of this is seen in the work on the nature and relative weight of the atom It is not appointed to every one to become a leading expert There are many good artists but only a few masters There are many notable workers in research, but only a few Curies, Ramsays, Millikans, Rutherfords, and Richards It is as much method and delicacy of manipulation as vision and initiative that make the master

This revision of methods improves the value of the instruments in the hands of the seeker of new facts and the explanation of new laws

HARVEY W WILEY

PREFACE TO FIRST EDITION

In presenting this revision of the official and tentative methods of analysis of the Association of Official Agricultural Chemists, it is proper to give a brief statement of the organization of the association, its purpose, and the procedure by which the methods are adopted

Membership in the association is institutional and is limited to the Departments of Agriculture, the State Agricultural Colleges and Experiment

ment Stations, the Federal Department of Agriculture, and the Federal, State, and City offices charged with the enforcement of food, feed, drug, fertilizer, insecticide and fungicide control laws

The association was founded at Philadelphia, Pa , September 9, 1884, by the following representative agricultural chemists of that time, the organization being the result of a series of informal meetings held the immediately preceding years

Prof H W Wiley, Chemist of the Department of Agriculture, Washington, D C
Mr Clifford Richardson, Assistant Chemist of the Department of Agriculture, Washington, D C
Mr Philip E Chazal, State Chemist of Virginia
Dr Chas W Dabney, Jr , State Chemist of North Carolina
Dr W J Gascoyne, State Chemist of North Carolina
Dr E H Jenkins, Connecticut Experiment Station
Prof John A Myers, State Chemist of Mississippi
Prof H C White, State Chemist of Georgia
Mr C DeGhequier, Secretary National Fertilizer Association
Dr Schumann, Dr Lehmann, Mr Gaines and others

At the first meeting methods for the determination of ammonia, phosphoric acid and potash in commercial fertilizers were adopted and work was begun for the perfection and adoption of methods for the entire range of agricultural chemistry Later the passage of food and drug and insecticide and fungicide control legislation by the States and by the Federal Government made it necessary to extend the scope of the association's activities for reason that the association methods were designated as the official methods for the enforcement of such legislation as well as for the control of feeds and fertilizers by the various states

To attain the aims of the association for a set of accurate methods, a system was evolved by which the methods in question are subjected to the most rigorous and painstaking scrutiny before they can be adopted A "referee" is appointed for any subject for which the association has not yet an official method or for a method which seems to require further investigation The referee conducts analyses according to the methods suggested for adoption in comparison with methods already established, obtaining the collaboration of as many as possible of the workers in that field In addition, a great deal of original research has been inaugurated on new methods This system developed logically until at the present time, in order to be adopted as "tentative," a method must be recommended to the association by the referee, and such recommendation is made only after the method has undergone a thorough collaborative and critical study Further, the special committee on methods must approve

the recommendation and the method must be accepted by a vote of the association In order to become "official," a method must be again accepted at another annual meeting The recommendations of referees are published in the reports of the proceedings of the association in the *Journal of the Association of Official Agricultural Chemists*, so that all tentative methods are made public before being adopted This permits consideration and criticism by chemists who are not members of the association It is immediately apparent that a method can be made official only after the most thorough series of tests not alone for accuracy, but for ease of operation as well It may be stated without reservation that more elaborate and painstaking effort has been expended on this collection of analytical methods than upon any other set of similar methods in the field of chemical science

The compilation and revision of the methods presented in this book was made by a committee of the association, consisting of R E Doolittle (chairman), B L Hartwell, G W Hoover, A F Secker (deceased), J P Street, and W A Withers Later, on the resignation of J P Street, A J Patten was appointed a member of the committee and the work of revision was continued

A preliminary revision, antedating the revision published in this book was printed in 1916 as supplementary parts to Volumes I and II of the *Journal of the Association of Official Agricultural Chemists* In this preliminary revision the committee received important assistance from R L Emerson, F C Blanck, and N A Parkinson At that time the scheme of numbering the sections in each chapter was adopted in order to simplify the system of cross-references

In the preparation of the present revision J A MacLaughlin rendered valuable assistance Acknowledgment is also made to the Library of the Department of Agriculture for assistance

Throughout its work, it has been the aim of the committee not only to bring the methods up to date, but especially to state the procedure with such lucidity and in such detail as to make it possible for any trained chemist to operate without being in doubt at any time

The work of the committee has been one of critical revision, compilation and editing The work of developing the methods was done by the various referees and their collaborators who have reported to the association at its annual meetings during the last decade To them is due the credit for the subject matter of this book

C L Alsberg
Secretary of the Association of Official Agricultural Chemists

September 17, 1920

INTRODUCTION TO FIRST EDITION

By Dr Harvey W Wiley, Honorary President of the Association of Official Agricultural Chemists

In the present edition of the methods of analysis, official and tentative, of the Association of Official Agricultural Chemists, the technic of analytical procedures has been revised to November 1, 1919 The monumental work of the Association of Official Agricultural Chemists is not only well known in the profession in this country, but is recognized in all countries as being the last word in agricultural chemical technic *The methods of determining the composition of agricultural products*, as well as of all bodies related to agriculture, has been recognized also by the courts of this country In case of judicial proceedings where different methods of analysis have been employed, the court, in all cases where the question has arisen, has recognized the official methods as binding

At the time of the organization of this body, referred to in the Preface, agricultural methods of research, from the chemical point of view, were extremely chaotic The progress of agricultural science which has marked its history in the last third of a century could not have been maintained amid these chaotic conditions The methods adopted by the founders of this association for correcting this state of affairs have been shown by experience to be the best possible I can say that the improvement in agricultural chemical technic has almost kept pace with the growth of the association

The gradual incorporation in the membership of the association of those scientific men engaged in the control of foods and drugs has widened the scope without altering the purpose of the original founders Today we find a body of scientific workers in agriculture and related subjects numbering quite half a thousand, who, by their activities and collaboration, have contributed to the pages of this volume, directly or indirectly The scientific knowledge of agriculture which has been verified and extended by this association now forms the foundation of all agricultural improvement

The profession of agriculture is the fundamental industry of this country Everything which strengthens the foundations of this industry benefits the country at large Our workers are not banded together for personal preferment, either in wages or in authority They have united for the sole purpose of benefitting agriculture and thus increasing production They

have not asked for shorter hours, nor for higher pay They have worked in season, out of season, by day and by night, on work days and holidays to perfect that science which, in its application, is the most powerful factor in scientific agriculture

The ability of the agricultural industry to withstand the assaults which are made upon it at the present time is largely due to the successful efforts of our association The agricultural industry has been built upon a rock and thus it is able to withstand the winds and the floods This industry is now in a more critical condition than any other The allurements of the city and the high wages of labor therein, have drawn from the farm much of its best blood and energy Congregate life has become so much more attractive than discrete life that it is hard to keep the young of both sexes upon the farm Yet it is plain that if man power and woman power upon the farm now be depleted the industry must suffer Making the farm attractive does not merely mean beautifying the house in which the farmer lives, making it more sanitary, planting trees, flowers and shrubs, but it means also the best knowledge of the soil and its properties, the most scientific data respecting the manufacture and use of fertilizing materials the most accurate knowledge of the character of crops best suited to the soil, and the best system of rotation which will help develop from the soil its most generous contribution In other words, not only must the farmer's farm be attractive and sanitary, but it must also be productive and dividend paying

We can well imagine the worth of the work which our association has done by picturing for a moment what the present agricultural industry would be if all that our science has contributed to it were stricken from human records In such a deplorable condition starvation would surely be staring the world in the face In the quiet corners of the laboratory, by the midnight oil and by personal devotion, the means which enable the farmer to get more remunerative crops have been worked out and perfected These workers, male and female, who have done this gigantic task have never been heralded in the public press, nor received encomiums of an admiring world They have done their work silently and effectively, without expectation of praise and without hope of pecuniary reward Their real reward has been in the consciousness of duty done The referees who have presided over this great work for the past thirty-six years and those who have aided them in these investigations, merit the generous regard and esteem of the whole scientific world, as well as the whole agricultural world Our association has been not one of debate nor of visionary plans of human welfare, but rather of hard work and concentrated devotion to the cause

The volume which is now laid before you contains the very last word of all that is important in agricultural research from the chemical and

physical point of view This does not mean that the field is fully exploited The great unknown of tomorrow doubtless holds in its secret embrace even greater prospects for human betterment than the days which have already passed This association stands ready and with expectant breath to receive the messages of tomorrow and translate them to the agricultural world

Washington, D C,
September 15, 1920

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ILLUSTRATIONS

DEFINITION OF TERMS AND EXPLANATORY NOTES

(1) The term "water" used in the methods means distilled water

(2) The reagents listed below unless otherwise specified have the approximate strength stated and conform in purity with the requirements of the United States Pharmacopoeia.

Sulfuric acid	Specific gravity 1 84
Hydrochloric acid	Specific gravity 1 184
Nitric acid	Specific gravity 1 42
Fuming nitric acid	Specific gravity 1 50
Glacial acetic acid	Specific gravity 1 048 (25°)
Hydrobromic acid	Specific gravity 1 38
Phosphoric acid	85 per cent strength by weight
Ammonium hydroxide	Specific gravity 0 90

(3) All other reagents and test solutions unless otherwise described in the text, conform to the requirements of the United States Pharmacopoeia or of the American Chemical Society When the anhydrous salt is intended it is so stated, otherwise the salt referred to is the crystallized product

(4) In the expressions (1—2) (5+4) etc , used in connection with the name of a reagent the first numeral indicates the volume of the reagent used and the second indicates the volume of water For example hydrochloric acid (1+2) means reagent prepared by mixing one volume of hydrochloric acid with two volumes of water When one of the reagents is a solid the expression means parts by weight, the first numeral representing the solid reagent and the second numeral the water

(5) In making up solutions of definite percentage it is understood that x grams of substance is dissolved in water and made up to 100 cc Although not theoretically correct, this procedure will not result in any appreciable error in any of the methods given in this book

(6) For the sake of simplicity the abbreviations Cl and I instead of Cl_2 and I_2 are used for chlorine and iodine Similar abbreviations have been used in other cases

(7) Recommendations to delete certain official methods were approved by the association at the 1930 meeting and first action for the deletion of such methods was taken Before the methods are finally deleted, however a second action must be taken The text of such methods is not printed in this edition but may be found in the 2nd (1925) edition of *Methods of Analysis* All official methods thus approved for deletion are marked with an asterisk unless a fuller explanatory note is given in connection with the method

(8) All calculations are based on the table of international atomic weights, Table I, under XLII

(9) The table for the conversion of specific gravity at 20/4° into alcohol is given in Methods of Analysis A O A C 1925 page 404 Action will be taken at the 1931 meeting of the association to make 15 56/15 56° 20/20° and 25/25 official in place of 20/4° Wherever directions are given to determine specific gravity at 20/4° it is advisable to use the temperatures given above Tables for the conversion of specific gravity into alcohol at these temperatures are given in this edition

Official and Tentative Methods of Analysis

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

I SOILS—TENTATIVE

DIRECTIONS FOR SAMPLING

1 (In view of the variability in soils it seems impossible at the present time to devise an entirely satisfactory method for sampling. It is obvious that the details of procedure should be determined by the purpose for which the sample is taken.)

Remove from the surface all vegetable material not incorporated with the soil. Take a sufficient number of samples to insure a composite sample that will be representative of the tract sampled, to the average depth of plowed soil, usually about 7 inches, and also take a composite sample from each important and distinctly different soil stratum to the depth of 40 inches, using a soil tube or auger. If a soil auger is used before boring below the plowed depth enlarge the first boring and carefully clean out the hole to prevent contamination of the successive sub strata while with drawing the samples. The sampling should be done when the soil is reasonably dry. Thoroly mix the samples of each depth and dry in a well ventilated, cool place.

In order to calculate the percentage results obtained by analysis to pounds per given area of the soil, determine the weight of a given volume of the soil as it lies in the field.

PREPARATION OF SAMPLE

2 (a) Reduce any soil lumps in the air-dried soil by rubbing in a porcelain mortar or by any other equally effective method that will not reduce the rock fragments and pass thru a sieve having circular openings 1 mm in diameter. Thoroly mix the sifted material and preserve in a suitable stoppered container. Weigh and discard the detritus.

(b) If necessary for the determination of the total quantity of any constituent, prepare more finely a sub-sample of (a).

If deviations from this procedure are deemed necessary, record them with the results.

MOISTURE

3 Dry 2 g of the sample prepared as directed under 2 (a), in a wide-mouthed weighing bottle at 100° to constant weight. Report the loss in weight as percentage of the moisture-free weight of the sample taken.

LOSS ON IGNITION

4 (This is useful as giving only a rough approximation of the organic matter and is not at all accurate for soils containing much combined water.)

Ignite the soil from 3 or 4 g in a platinum or other suitable substitute to full redness, stirring occasionally until the organic matter is destroyed. If the soil contains appreciable quantities of carbonates moisten after ignition with a few drops of a saturated solution of

$(NH_4)_2CO_3$, dry, heat to dull redness to expel the NH_4 salts, cool in a desiccator, and weigh Report the percentage loss in weight as organic matter

CARBONATE CARBON

5

APPARATUS

Quadruplicate shaking apparatus for evolution of carbonate carbon in soils of high or low carbon content [2]—This apparatus (Figs 1 and 2) consists of a horizontal holder (*H*) 21 inches long, $\frac{7}{8}$ inch thick, and $1\frac{3}{4}$ inches wide, having properly spaced slots

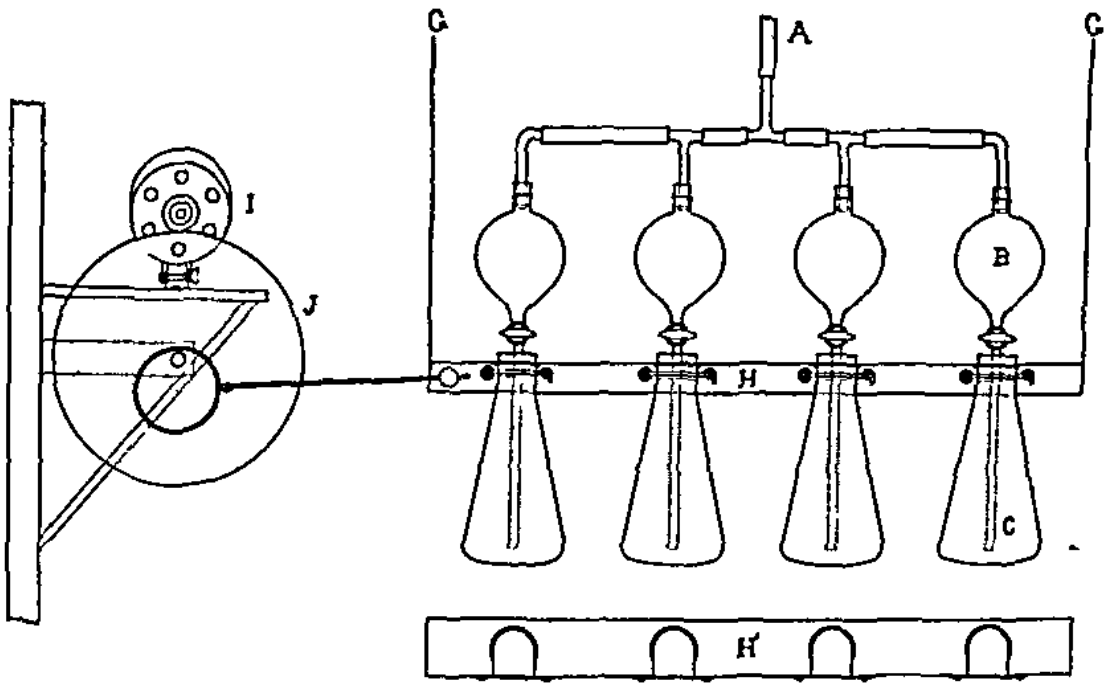

FIG 1—QUADRUPLICATE SHAKING APPARATUS FOR DETERMINATION OF CARBONATE CARBON IN SOILS

made to fit loosely the neck of a 300 cc Erlenmeyer flask taking a No 6 rubber stopper The holder (*H*) is suspended horizontally from a bar by means of brass strips $1\frac{1}{4}$ inches wide and 24 inches long The common intake for purification of the incoming air leads from a tube about 25 inches long

This tube stands upright, extending thru a rubber stopper in a 1 liter Erlenmeyer flask, and to prevent the mechanical carrying over of any of the purifying NaOH it has inserted in the top a large *N* distillation bulb

The driving wheel (*J*) is $\frac{1}{4}$ inch thick and 7 inches in diameter The eccentric attached to its face is $\frac{1}{2}$ inch in thickness and 2 inches in diameter This eccentric is grooved to permit free rotation of the driving shaft, which is fastened to the end of the holder (*H*) by means of a binding post Power for agitation is supplied by the motor (*I*), a sewing machine, or small desk fan motor If the motor available has no rheostat, its speed can be easily controlled by a battery of 4 lamps The motor is hinged upright on the support so that the pulley will rest upon the edge of the driving wheel To reduce noise the pulley of the motor is inserted into a rubber stopper Or the driving wheel (*J*) may be made to carry a belt that is driven by the pulley of a small motor

The absorption towers (*D*) are at least 25 inches high and 1 inch in diameter They contain alternating pockets of solid glass rods and small glass beads resting upon an inverted test tube $2\frac{1}{2}$ inches long The rubber connection on the intake cock of the

tower (*D*) is u ed to disconnect the glass tube that extends to the rubber connection on the safety bulb tube leading from flask (*C*)

DETERMINATION

6 *Method I—Volumetric*[2]

Pulverize the sample to pass a 60-mesh sieve, so as to expose fully to the action of the liberating acid any calcite that may be included in quartz crystals For soils low in carbonates use a 10 , 25 or 50 g charge in the quadruplicate shaking device described under 5

Introduce the charge into the 300 cc evolution flask (C Figs 1 and 2) aspirate 5 min to free the apparatus of atmospheric CO_2 release the suction and then introduce 10 25 or 50 cc of 0 5 *N* NaOH or KOH soln into the absorption tower Apply a suction of 5 inches and then introduce 60 cc of HCl (1+9) containing 5 g of $SnCl_2$ upon the soil contained in the 1 rlenmeyer flask, regulating the intake of air by means of a screw cock placed just beyond the absorption tower Agitate and aspirate for 60 min at the rate of 3-4 bubbles per second Then relea e the suction and draw off the absorbent soln into a 500 cc flask washing the tower with a succession of fillings of CO_2-free H_2O to a volume of 150 cc Add 10 cc of neutral aqueous soln of $BaCl_2$ (made by dissolving 250 g of $BaCl_2 \cdot 2H_2O$ in 1 liter of H_2O) make to volume, agitate and allow to stand 4 hours Titrate the excess of hydroxide, using phenolphthalein indicator With a small bore buret permitting split-drop readings to hundredths of a cc use 0 2 *N* acid for the titration with burets of larger bore use 0 1 or 0 2 *N* acid Calculate and report the result as per centage of carbonate C or CO_2

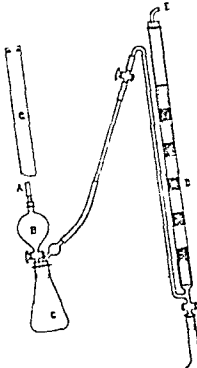

FIG 2—ABSORPTION TOWER

7 *Method II—Gravimetric*

Proceed as in 6 but in lieu of the NaOH and KOH absorbent use an absorption tube filled with a carrie This tube should be preceded in the train by tubes containing an Ag SO_4 suspension in H_2SO_4(1+19), H_2SO_4 and $CaCl_2$ in order Report the percentage on the weight found for the ascarite tube in percentage of carbonate C or CO_2

NOTE—Special consideration should be given to the soils that have been treated with magnesium chloride or those known to be derived from the limited areas of rocks in the glaciated region where transported dolomite may occur In these cases add equivalent quantities of each acid to the soil suspension until no CO_2 evolution is noted by the gain in weight of the absorption train Remove heat developed by the acid reaction by cooling the flask in a bath of ice water Then aspirate as in 6 Draw CO_2-free air thru the apparatus for 20 min The absorption may be accomplished by either the volumetric (6) or the gravimetric (7)

Method III—Furnace Combustion Method[3]

8 APPARATUS

(a) *Oxygen cylinder* —With pressure regulating valve

(b) *Electric combustion furnace* —With rheostat, and with $\frac{7}{8}$ x 24 inch fused silica tube containing an 8 inch loosely packed core of platinized asbestos (CuO may be used if a temp of 950° is not exceeded)

(c) *Purification and absorption train* —Place 2 scrubber bottles containing a 10% soln of KOH, followed by an Hg valve between the O supply and the intake end of the furnace Provide an asbestos-filled Cu coil with handle as an insulating plug on the intake end of the combustion tube, also use an asbestos plug to insulate the rubber stopper at the outlet end of the combustion tube The outcoming current is dried and purified by an H_2SO_4 scrubber, a tube containing 40 mesh granulated Zn and a tube of P_2O_5, or equivalent drying material in order The drying tube is connected with a Nesbit absorption bulb filled with alternate layers of glass wool and ascarite The Nesbit tube is protected against moisture and back pressure at its outlet by a Fisher bubble counter containing concentrated H_2SO_4

9 DETERMINATION

Bring the furnace to a temp of 900–950 Connect the train and sweep out the apparatus by an adjusted flow of O Weigh the Nesbit tube against a counterpoise Replace the Nesbit tube in the train and introduce well within the heated zone an alundum boat containing a 2 g charge of soil admixed with 2 g of finely divided CuO Close the intake and open the Nesbit bulb When no further flow of gas passes thru the absorption train, connect with suction, adm t a flow of O and aspirate for 30 min Close the Nesbit tube, disconnect, and weigh against the counterpoise Correct the total evolution of CO_2 for the carbonate CO_2 (determined as 6 or 7) and report as organic C, or CO_2 There should be no carbonates in the residue in the case of acid, calcareous or dolomitic soils Unleached alkali soils should be corrected for the original and any residual CO_2

ORGANIC CARBON

Wet Oxidation Method

10 REAGENTS

(a) *Oxidizing soln* —Dissolve 85 g of CrO_3 in 100 cc of H_2O and make to 250 cc with 85% H_3PO_4

(b) *Acid soln* —Mix equal volumes of 85% H_3PO_4 and boiled concentrated H_2SO_4

11 DETERMINATION

Introduce 1–5 g of soil, depending upon the organic matter content, into each of the four 300 cc Pyrex Erlenmeyer flasks, 5 In addition, insert a glass bulb of about 1½ inches diameter between the Erlenmeyer flasks and the absorption towers and bend the tube leading from this bulb into the Erlenmeyer flask so as to permit the return of the condensed H_2O along the side of the flask In order to guard against the mechanical aspiration of the liberating acids place an empty absorbent bead filled tower between each glass bulb and the tower that contains the hydroxide absorbent Free the apparatus of atmospheric CO_2 and then introduce into each absorption tower 25 or 50 cc of 0 5 N NaOH or KOH Apply suction of 5 inches and run into each Erlenmeyer flask 10 cc of the oxidizing soln Then add 25–10 cc of the acid mix

ture Gently agitate the flasks and place a low flame under each Continue the gentle agitation and heating for 30 min after the mixture begins to boil

At the end of the agitation and aspiration, release the suction and wash the absorbent into a 500 cc flask Precipitate the Na_2CO_3 or K_2CO_3 by adding 10 cc of a neutral aqueous soln of $BaCl_2$ (250 g of $BaCl_2$ $2H_2O$ per liter) Dilute to volume and permit the precipitate of $BaCO_3$ to settle 4 hours Pipet an aliquot of 200 cc and titrate the residual hydroxide with 0 5 *N* acid, using phenolphthalein indicator and splitting drops by the use of a stirring rod near the end of the titration

Residual hydroxide in terms of 0 5 *N* alkali minus the 0 5 *N* NaOH originally used = the CO_2 present in the sample Total CO , as determined under 6 = percentage of carbonate C or CO_2 derived from the oxidation of the organic material

TOTAL NITROGEN

12 *Gunning Hibbard Method*

Digest 10 g of soil in a 500 cc Kjeldahl boiling flask with 30–40 cc of concentrated H_2SO_4 and approximately 10 g of salt mixture composed of 10 parts of K_2SO_4 or anhydrous Na_2SO_4 1 part of $FeSO_4$ and ½ part of $CuSO_4$ Continue the digestion until the mixture is colorless or nearly so After cooling, dilute the contents of the flask with H_2O add an excess of an approximately 45% NaOH soln connect with the condenser and distil 150 cc into standard acid as directed under II, 19 The distillation may be carried out in the digestion flask or, if preferred the soln may be transferred to a Cu flask Titrate the excess of acid with 0 1 *N* or *N*/14 alkali using methyl red or cochineal indicator Report as percentage of N

13 *Kjeldahl Method*

Proceed as directed under 12, using 0 7 g of HgO or 0 65 g of Hg instead of the salt mixture Mix immediately and heat over a low flame, gradually increasing the heat Continue the digestion until the mixture is colorless or nearly so After cooling dilute the contents of the flask add 25 cc of sulfide or thiosulfate soln {II, 17 (h)} and an excess of the NaOH soln, and proceed with the distillation and titration as directed under II, 19 Report as percentage of N

SODIUM CARBONATE FUSION[a]

14 *Method I*

Thoroly mix on glazed paper 5 g of soil ground to an impalpable powder, with 25 g of Na_2CO_3 and transfer carefully to a 100 cc Pt or Ni crucible Cover, heat at low redness until fusion begins then increase the heat until a clear, quiet fusion results, finally, give full heat of a Meker burner for 20 min having the flame oblique to insure good oxidation Pour the fusion into a large Pt dish set in water Place the crucible and cover in a wide 400 cc beaker Cover with water transfer the fused lump from the Pt dish to the beaker and rinse the dish into the beaker with HCl (1+9) Add 50 cc of HCl to the contents of the beaker, cover and keep on a steam bath until the fused mass has disintegrated Transfer the mixture to the Pt dish and evaporate to dryness on a steam bath

15 *Method II*

Proceed as directed under 27

16

SILICA

Take up the residue from 14 or 15 in HCl (1+9) and filter the mixture so obtained (a 9 cm Büchner funnel with suction may be used advantageously) Wash with hot

H_2O containing 5 cc of HCl per liter Collect the filtrate and washings in a dish, preferably a casserole, and dehydrate on a steam bath until the SiO_2 assumes a crystalline appearance Moisten with HCl and repeat the dehydration for 2 hours Add 5 cc of HCl and 100 cc of hot H O Mix thoroly, filter, and wash Add the residue to the main portion of SiO_2 obtained from the first filtration Make up the combined filtrate and washings to 500 cc at 20° and save for subsequent determinations Place the two SiO_2 residues with filters in a porcelain crucible Moisten with a saturated NH_4NO_3 soln Ignite with low heat at first to burn off filter paper and then with a strong flame, preferably a blast lamp, to constant weight, cool in a desiccator, and weigh Report as percentage of SiO_2

17 OXIDES OF IRON ALUMINUM MANGANESE, PHOSPHORUS AND TITANIUM

To a 100 or 200 cc aliquot of the soln from 16, according to the probable quantity of Fe and Ca present, add NH_4OH (1+1) dropwise until the precipitate formed requires several seconds to dissolve, thus leaving the soln faintly acid Add 0 5 g of solid NH_4 persulfate, heat nearly to the boiling point, and add sufficient NH_4OH (1+1) to precipitate all Fe, Al, etc Allow the mixture to boil in a covered beaker for about 1 min , remove, and if no NH_3 is given off (as detected by smelling), again add NH_4OH dropwise until it can be detected Do not allow the precipitate to settle, but stir and pour on the filter Wash immediately with hot 2 5% NH_4NO_3 soln, playing a fine jet around the edge of the precipitate, thus cutting it free from the paper in order to insure rapid filtration Wash the precipitate several times Transfer the paper and the precipitate to the original beaker Add 5 cc of HCl, macerate quickly, add 50 cc of H_2O, and heat (This procedure insures that the paper will not be carried to the gel state) Reprecipitate the oxides with NH_3 persulfate and dilute NH_4OH as directed above, filter, and wash until free from chlorides with hot 2 5% NH_4NO_3 soln Reserve the filtrate and washings from both *the first and second precipitations for the determination of Ca and Mg*

Dry the precipitate, remove from the filter, and ignite in a Pt crucible over a Bunsen flame, incinerating the filter separately, and add the residue to the precipitate Then ignite to bright redness, cool in a desiccator, and weigh as Fe_2O_3, Al_2O_3, Mn_3O_4 TiO_2, and P_2O_5

To this residue add $KHSO_4$, or $K_2S_2O_7$, and heat at low temp until the precipitate is completely disintegrated, cool quickly and transfer to a flask containing 100 cc of H_2SO_4 (1+3) Dissolve the melt, reduce with Zn, cool, and determine Fe by titration with 0 2 *N* $KMnO_4$ soln (cf XXXVII, 58) Report as percentage of Fe_2O_3

Or, in lieu of the above fusion, evaporate 50 or 100 cc of the soln from 16 after the addition of 10 cc of H_2SO_4 until all HCl is expelled Dilute with H_2O, reduce with Zn, and determine the Fe by titration with 0 2 *N* $KMnO_4$ soln

Evaporate 50–100 cc of the soln from 16 after the addition of 10 cc of HNO_3 Repeat the addition of HNO_3 and evaporation to insure expulsion of all HCl From this point proceed as directed under XXXVII, 75 Subtract the sum of the oxides of Fe, Mn, and P (determined separately as directed in 29 or 30) from the weight of the combined oxides of Fe, Al Mn, P, and Ti, determined as directed above Report the remainder as oxides of Al and Ti

18 CALCIUM

Concentrate the combined filtrates and washings from 17 to about 50 cc, make slightly alkaline with NH_4OH (1+1), and add, while still hot saturated NH_4 *oxalate soln dropwise as long as any precipitate is produced and then an excess* sufficient to convert the Mg salts also into oxalate Heat to boiling allow to stand 3 hours or longer decant the clear soln thru a filter, pour 15–20 cc of hot H_2O on the

precipitate, and again decant the clear soln thru the filter Dissolve the precipitate in the beaker with a few drops of HCl, add a little H_2O, and reprecipitate, boiling hot, by adding NH_4OH and a little NH_4 oxalate soln Allow to stand as before and filter thru the same filter Wash free from chlorides with hot H_2O Reserve the filtrates and washings from both precipitations for the determination of Mg under 19 or 21 Complete the determination by one of the following procedures and report as percentage of CaO

(a) Ignite the precipitate in a crucible over a S free blast to constant weight, cool in a desiccator, and weigh the CaO

(b) Incinerate the filter over a low flame, mix the ignited precipitate with a finely pulverized and dried mixture of equal parts of $(NH_4)_2SO_4$ and NH_4Cl, and drive off the excess of the sulfate by careful heating of the upper portion of the crucible Complete the ignition, cool in a desiccator, and weigh the $CaSO_4$[a]

(c) Dissolve the Ca oxalate precipitate from the filter with hot H_2SO_4 (1+1), collect the soln in the beaker employed for precipitation, and titrate while hot with a standard soln of $KMnO_4$ (cf XII, 9) Finally add the filter paper unmacerated to the soln and complete the titration

MAGNESIUM

Method I

19 Evaporate the combined filtrates and washings from 18 on a water bath to about 100 cc and add cautiously 20 cc of HNO_3 Evaporate to dryness and heat carefully on a hot plate to remove NH_4 salts Add 5 cc of HCl and evaporate nearly to dryness Dissolve the residue in hot H_2O and a small quantity of HCl If necessary, filter the soln and wash the filter paper with about 100 cc of hot H_2O Precipitate the Mg as $MgNH_4PO_4$ by the addition of 3 cc of a 10% soln of NH_4 phosphate and sufficient NH_4OH to make the soln slightly alkaline Stir the soln vigorously allow to stand 15 min, add 15 cc of NH_4OH, and allow the precipitation to proceed overnight Filter, wash the precipitate with NH_4OH (1+9), transfer to a porcelain crucible ignite cool, and weigh as $Mg_2P_2O_7$ The filtration may be made thru a Gooch crucible Calculate and report the result as percentage of MgO

Method II[a]

REAGENTS

20 (a) *Sodium ammonium phosphate soln*.—Dissolve 100 g of $NaNH_4HPO_4\ 4H_2O$ in hot H_2O cool, and dilute to 1 liter

(b) *Dilute ammonium hydroxide for washing*.—Dilute 100 cc of NH_4OH to 1 liter

DETERMINATION

21 To the combined filtrates and washings from 18 add 100 cc of NH_4OH and 50 cc of 95% alcohol Then add with constant stirring 25 cc of the $NaNH_4HPO_4$ soln and let stand 12–24 hours Filter, wash twice with the dilute NH_4OH and dissolve the precipitate in HNO_3 (1+4) washing the soln into the original beaker to a volume of 100–150 cc To this soln add ½ volume of NH_4OH, 25 cc of 95% alcohol and 2 drops of the Na NH_4-phosphate soln Stir vigorously and allow to stand 3 hours or longer Filter thru a Gooch crucible, wash with the NH_4OH ignite and weigh as $Mg_2P_2O_7$ Calculate and report the result as percentage of MgO

MANGANESE

Method I

REAGENTS

22 The reagents and solns used are described under XXXVII, 74

23 DETERMINATION

Treat 1 g of 100 mesh soil with 5 cc of HF and 5 cc of H_2SO_4(1+1) Evaporate to dryness, ignite, and fuse the residue with $KHSO_4$ Repeat the addition of HF until all silicates are decomposed Dissolve in H_2O, add HNO_3 and evaporate to dryness Again dissolve in H_2O, add 25 cc of HNO_3 (1+2), then add about 0 5 g of Na bismuthate, and heat until the permanganate color disappears From this point proceed as directed under XXXVII, 75, beginning with 'Add a few drops of a soln of NH_4 or Na bisulfite to clear the soln' Report as percentage of mangano manganic oxide (Mn_3O_4)

Method II[7]

24 REAGENTS

(a) *Sulfuric acid soln* —(1+1)

(b) *Potassium bisulfate* —Mn free and finely pulverized

(c) *Standard manganous sulfate soln* —Dissolve 0 2877 g of pure $KMnO_4$ in about 100 cc of H_2O, acidify the soln with H_2SO_4 (1+1), and slowly heat to boiling Add slowly a sufficient quantity of a 10% oxalic acid soln to discharge the color Cool, and dilute to 1 liter 1 cc of this soln = 0 1 mg of Mn

(d) *Potassium periodate*

25 DETERMINATION

Weigh 0 5–5 0 g of finely pulverized air dried soil into a 50 cc SiO_2 or Pt crucible Add to the soil approximately 2½ times its weight of finely powdered, Mn free $KHSO_4$ and mix thoroly Place the lid on the crucible and heat gently over a Bunsen burner about 5 min , increase the heat gradually until the crucible and lid are red hot, being careful not to allow the contents of the crucible to froth over Continue to heat for about 20 min or until the frothing has ceased and the contents are in a quiet molten condition Withdraw the flame from beneath the crucible, remove the lid, and rotate the crucible in a horizontal position to spread the molten contents over the inner walls to expedite cooling When the crucible is no longer red, immerse it in about 25 cc of H_2SO_4 (1+1) in a 250 cc beaker and digest on a hot water bath until the contents of the crucible disintegrate and dissolve Carefully rinse the crucible and lid with hot H_2O and dilute the soln to about 100 cc Filter, and wash the insoluble residue

Discard the insoluble residue if it has a uniform white color, if it is colored by undecomposed particles of minerals ignite and expel the SiO_2 with HF and H_2SO_4 Fuse the residue with $KHSO_4$ digest in dilute H_2SO_4 and add the soln to the filtrate from the fusion

Make the soln to a definite volume, take an aliquot for the determination, and add about 0 05 g of K periodate to the aliquot Boil the soln until the characteristic purplish permanganic acid color develops, heat on a hot water bath for an hour, and set aside to cool If the color is deep purple, dilute the soln to a definite volume Remove an aliquot and match against a standard Mn soln in Nessler jars or in a colorimeter Compute the results as percentage of Mn or Mn_3O_4 (A series of Mn standard solns are prepared from reagent (c) by removing aliquots of the $MnSO_4$ soln and developing the Mn color with K periodate in the same way as the soln of the sample

26 IODINE[8]

Weigh 50 g of air-dried soil into a quartz boat and introduce into the quartz combustion tube of an electric furnace Attach 3 absorption bottles containing thin

suspensions of $Ca(OH)_2$ and connect with suction apparatus To the other end of the furnaces attach 2 bottles containing 5% KOH soln Heat the furnace to a temp of 1100° and aspirate with I free air for 2 hours Cool wash the contents of the $Ca(OH)_2$ absorption train into a large beaker and digest for 30 min Filter and wash thoroly Transfer the filtrate to a porcelain dish and evaporate to dryness Moisten the residue with a few cc of hot H_2O transfer to a small quartz dish, add 2 cc of a $Ca(OH)_2$ suspension, evaporate to dryness and ignite gently to remove traces of organic matter Cool moisten the ignited residue with hot H_2O and filter to a volume of 10 cc in a 30 cc separatory funnel Add 1 cc each of CS_2, H_2SO_4 (1+9) and 1% $NaNO_2$, stopper tightly and shake vigorously for 1 min Test an aliquot of a standard KI soln similarly in a second funnel Compare the pink colors and compute the values in terms of parts per billion of soil

SULFUR[1]

PREPARATION OF SOLUTION

27 Weigh 5–10 g of the soil, prepared as directed under 2(b) to pass a 0.5 mm sieve into a 100 cc Ni crucible add an equal weight of anhydrous Na_2CO_3, and mix well with a stout Ni stirring rod of such length as to permit introduction into the furnace to be used in the fusion Pipet carefully 4 cc of H_2O into each 10 g of soil, stir well to a stiff paste adding more H_2O if necessary a few drops at a time Immediately add succe sive portions of about 1 g of S free Na_2O_2, stirring well after each addition to obviate excessive frothing and overflow Continue to add peroxide until the mix ture becomes dry and granular and then add as a surface coating enough to make the total peroxide addition 2.5 g per each 10 of soil Place the mixture in an electric furnace maintain a temp between 400 and 500° during the first half hour then rai e it rapidly to bright red heat (about 900°) and continue the fusion at this temp for about 10 min Withdraw the crucible from the muffle quickly manipulate so as to cau e the melt to spread out in a thin sheet over the interior of the crucible and cool rapidly by contact with some good conductor in a cool atmosphere Place the chilled crucible sideways in a 600 cc beaker and cover with H_2O Add about 5 cc of 95% alcohol to decompose the Na manganate Cover the beaker with a watch glas s place on a cold hot plate and apply heat Boil briskly until all the melt is disintegrated (30 min is ordinarily sufficient) When the suspen ion has assumed a fle h-colored flocculent appearance with no glassy green lumps in the interior of the crucible remove the crucible and rod from the beaker and wash any flaky par ticles back into the beaker with the aid of a rubber tipped glass rod rin ing several times with hot H_2O (If small glassy particles still cling to the in ide of the crucible dis integrate by boiling H_2O over the hot plate or a small flame and add to the main portion) Filter immediately by suction thru a 9 cm Büchner funnel into a liter beaker placed under a bell jar When no more of the liquid can be drawn thru the filter return the residue together with the filter paper to the original beaker wash ing any adhering particles carefully from the funnel Add about 1 g of Na_2O_2 ma erate with the stirring rod add 100 cc of H O and bring to a brisk boil while stirring vigorously Again filter thru a Büchner funnel using suction until nearly dry and wa h with 50 cc portions of hot H_2O to a total volume of 500 or 700 cc for the 5 and 10 g charges respectively

DETERMINATION

28 Add 1 to 3 drops of wh and with stirring sufficient HCl to neutralize the e In ing methyl red indicator [II 17(k)] Add 0.5 cc excess of the HCl and con cen trate by heating to a vol ume of 150 cc Heat to boiling a dd slowly 10 cc of a 5%

$BaCl_2$ soln, and allow to stand overnight Filter on a dense filter paper, place paper in a Pt crucible, and ignite in an electric furnace Cool in a desiccator, and weigh as $BaSO_4$ To insure against possible inclusion of SiO_2, add 2 drops of HF and 1 drop of H_2SO_4 (1+1), heat carefully, reignite, and weigh Report as percentage of S or SO_3

PHOSPHORUS

29 *Sodium Peroxide Method*

Place 10 g of Na_2O_2 in an iron or porcelain crucible and thoroly mix with it 5 g of the soil prepared as directed under 2(b) If the soil has very little organic matter, add a little starch to hasten the action Heat the mixture carefully by applying the flame of a Bunsen burner directly upon the surface of the charge and the sides of the crucible until the action starts Cover the crucible until reaction is over and keep at a low red heat for 30 min Do not allow fusion to take place By means of a large funnel and a stream of hot H_2O transfer the charge to a 500 cc volumetric flask, acidify with HCl and boil Cool, and make up to the mark (If the action has taken place properly, there should be no particles of undecomposed soil in the bottom of the flask) Allow the SiO_2 to settle and draw off 200 cc of the clear soln

Or, in lieu of the above, oxidize 5–10 g of the material and disintegrate the melt as directed under 27 Acidify with HCl, dilute to 500 cc, and withdraw a 200 cc aliquot

Precipitate the Fe Al, and P with NH_4OH (1+1), filter, wash several times with hot H_2O, return the precipitate to the beaker, and dissolve it in hot HCl (1+4), pouring the acid upon the filter to dissolve any precipitate remaining Evaporate the soln and washings to complete dryness on a water bath Take up with HNO_3 (1+4) heating if necessary and filter to remove SiO_2 Evaporate the filtrate and washings to about 10 cc and add 2 cc of HNO_3 Neutralize the excess of acid with NH_4OH (1+1) and then add HNO_3 until the soln becomes clear, avoiding an excess of the acid Heat to 40–50° in a water bath, add 15 cc of molybdate soln [II, 5(a)] and keep at this temp for 1–2 hours Let stand overnight filter, and wash free from acid with cold H_2O Transfer the filter to a beaker and dissolve in standard NaOH or KOH (1 cc = 0 5 mg of P_2O_5) Titrate the excess of alkali with standard HNO_3, using phenolphthalein indicator Or, after adding the 15 cc of molybdate soln, allow to stand 3 hours at a temp not above 60°, filter on a small filter or on a Gooch crucible, and wash with cold H_2O until two fillings of the filter do not greatly diminish the color produced with phenolphthalein by 1 drop of standard alkali Return the filter and precipitate to the same beaker used for precipitating the phosphomolybdate dissolve the yellow precipitate in standard NaOH or KOH (1 cc = 0 5 mg of P_2O_5), add a few drops of phenolphthalein indicator and titrate the excess of alkali with standard HNO_3 Report as percentage of P_2O_5

30 *Magnesium Nitrate Method*

Place 5 g of soil, prepared as directed under 2(b), in a porcelain dish Moisten with 5–7 cc of $Mg(NO_3)_2$ soln [II, 5(e)] Dry on a water bath and burn off the organic matter at low redness Cool and add 10 cc of H_2O, 10 cc of HCl, and 5 cc of HNO_3 Cover the dish and digest the contents for 2 hours on a water bath, stirring 2 or 3 times during the digestion Dilute to 200 cc mix well and filter thru a dry folded filter pouring back thru the filter until the filtrate is clear Place an aliquot *corresponding to 2 or 1 g of the soil depending upon the quantity of P present* in a hard glass beaker or porcelain dish and evaporate to dryness on a water bath Take up with HNO_3 (1+4), again evaporate to dryness, and heat for 1 hour at 110–120

Again take up with the dilute HNO_3 and filter Reduce the combined volume of filtrates and washings to 30–40 cc Make alkaline with NH_4OH (1+1), and dissolve the precipitate with HNO, using a slight excess Add gradually with vigorous agitation 15 cc of molybdate soln [(II, 5(a)] Keep the soln at 40–50° for an hour and then let stand overnight at room temp Filter, and wash well with cold H_2O Return filter and precipitate to the same flask and determine P volumetrically as directed under 29 Report as percentage of P_2O_5

31 POTASSIUM AND SODIUM OR POTASSIUM ONLY

Triturate gently 0 5 or 1 g of the finely ground soil with 1 g of dry NH_4Cl in a smooth mortar, add 8 parts of $CaCO_3$, and mix intimately Transfer the mixture to a Pt crucible rinsing the mortar with a little $CaCO_3$ Heat the crucible gradually until fumes of NH_4 salts no longer appear and the lower ⅓ of the crucible is brought to a red heat Maintain this temp 40–60 min The temp should be sufficient to keep the $CaCl_2$ formed by the reaction of NH_4Cl with $CaCO_3$ in a state of fusion (The mass does not become liquid because the fused $CaCl_2$ is absorbed by the large quantity of $CaCO_3$ present If the silicate is fused by the application of too strong heat, disintegration of the mass at the end of the operation with H_2O cannot be effected Moreover, too high a temp causes volatilization of alkali chlorides The mass contracts in volume during the ignition and is usually easily detached from the crucible) Transfer the fused mass to a porcelain dish slake with hot H_2O and grind thoroly with an agate pestle After washing 5 times by decantation with hot H_2O, transfer to a filter and wash well (300 cc of wash water is sufficient) To the filtrate add sufficient $(NH_4)_2CO_3$ soln to precipitate the Ca and any Mg present Allow to settle, decant the supernatant liquid into a porcelain dish, and concentrate by evaporation, finally transferring the precipitate to the dish When the volume is reduced to about 30 cc add a little $(NH_4)_2CO_3$ and NH_4OH, heat, filter into a porcelain dish, evaporate the filtrate to dryness on a water bath, and expel NH_4 salts by ignition or evaporate with 10 cc of HNO_3 followed by 2 evaporations with 10 cc of HCl

If K alone is to be determined proceed from this point as directed under II, 43(a), beginning with "Dissolve the residue in hot H_2O Report as percentage of K_2O

If both K and Na are to be determined dissolve the residual alkali chlorides in 3–5 cc of H_2O a little black or brown flocculent matter usually remains undissolved, and the soln may also contain traces of Ca Add 2–3 drops of the $(NH_4)_2CO_3$ soln and NH_4OH, warm and filter thru a small filter into a weighed Pt dish Evaporate to dryness on a water bath carefully heat the residual alkali chlorides to incipient fusion, cool, and weigh as Na and K chlorides Dissolve the combined chlorides in 30 cc of H_2O, add 1 5 cc of $PtCl_4$ soln [II, 41(b)] evaporate to a syrupy consistency and add 15 cc of 2 25 N acidulated alcohol (prepared by passing HCl gas into a mixture of 2000 cc of 95% alcohol and 152 cc of HCl) Filter thru an asbestos Gooch and wash with the acidulated alcohol and then with 80% alcohol Dry the Gooch for one hour in an electric oven and then weigh Dissolve the potassium platinic chloride with hot H_2O, wash with 80 % alcohol and again place in a drying oven for an hour Cool in a desiccator weigh and calculate to KCl Calculate to KCl and deduct from the weight of combined Na- and K-chlorides to obtain NaCl Calculate and report the result as percentage of Na O

prepared as directed under 2(a), on the filter paper With a horn spoon or clean spatula press the soil down firmly against the paper and add enough H_2O (tested and found neutral) to saturate the soil Cover the dishes allow to stand for 30 min , and then note the color of the test paper A check Petri dish containing neutral litmus paper and filter paper, moistened with the same H_2O, is allowed to stand under the same conditions The filter paper gives a uniform background and evenness of contact

RE CTION VALUES

33 DETERMINATION[11]

Use either the colorimetric or electrometric method, as found convenient Determine the reaction values on fresh moist samples, using a soil to-water ratio of 1 5, with intermittent agitation for 30 min

METHOD OF STATEMENT

For simplicity and ease of interpretation use a dual system of statement, giving both pH values and their equivalents in arithmetrically related numbers in parentheses, as tabulated below

SØRENSEN OR pH VALUES	SPECIFIC ACIDITY OR HYDROGEN ION *CONCENTRATIONS*	SØRENSEN OF pH VALUES	SPECIFIC ALKALINITY OR HYDROXYL-ION *CONCENTRATIONS*
7 0	0 0	7 0	0 0
6 9	0 5	7 1	0 5
6 8	1 0	7 2	1 0
6 7	1 5	7 3	1 5
6 6	2	7 4	2
6 5	3	7 5	3
6 4	4	7 6	4
6 3	5	7 7	5
6 2	6	7 8	6
6 1	8	7 9	8
6 0	10	8 0	10
5 9	12 5	8 1	12 5
5 8	16	8 2	16
5 7	20	8 3	20
5 6	25	8 4	25
5 5	31 5	8 5	31 5
5 4	40	8 6	40
5 3	50	8 7	50
5 2	63	8 8	63
5 1	80	8 9	80
5 0	100	9 0	100
4 9	125	9 1	125
4 8	160	9 2	160
4 7	200	9 3	200
4 6	250	9 4	250
4 5	315	9 5	315

34 NITRATE NITROGEN

Place 100 g of the air dried soil prepared as directed under 2(a), and 500 cc of H_2O in a suitable container, and agitate for 5 min Add 1 g of CaO or 2 g of precipitated $CaCO_3$ agitate thoroly allow to stand 10–20 min and filter, making sure that a clear filtrate is obtained If the filtrate contains 6 parts of Cl per million or less proceed as directed under XXXVII, 17, using 25 cc of the filtrate, if it contains more than 6 parts of Cl per million, proceed as directed under XXXVII, 19, using 25 cc or a quantity that will contain not to exceed 0 1 mg of N in the form of nitrate Report as percentage of nitrate N

To 100 g of soil in a 500 cc bottle, add 250 cc of H_2O Stopper shake thoroly, and allow to stand overnight Filter thru a Pasteur Chamberland filter Evaporate 50 cc of the filtrate to dryness in a Pt dish on a steam bath, ignite at a low red heat to drive off organic matter, cool in a desiccator, and weigh for total salts Dissolve the residue in the Pt dish in 10–15 cc of hot H_2O, transfer to a 50 cc volumetric flask, cool, and dilute to the mark

For determination of Cl titrate an aliquot of 10 cc against 0 1 N $AgNO_3$ soln Calculate and report the result as percentage of NaCl

For determination of alkali carbonate, titrate an aliquot of 10 cc against 0 1 N HCl Calculate and report the result as percentage of Na_2CO_3

Determine sulfates by difference If much gypsum is present, filter the soln of the salts in hot H_2O thru a small filter, weigh the gypsum separately, and subtract the amount from the total amount of sulfates

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II FERTILIZERS

GENERAL METHODS

1

DIRECTIONS FOR SAMPLING—OFFICIAL

Each official sample sent to the laboratory shall consist of at least 1 pound of the material taken in the following manner Use a sampler that removes a core from the top to the bottom of the bag Take cores from not less than 10 per cent of the bags present unless this process necessitates cores from more than 20 bags in which case take a core from 1 bag for each additional ton represented If less than 100 bags, sample not less than 10 bags, if less than 10 bags, sample all bags Thoroly mix the portions taken on a clean oilcloth or paper, reduce by quartering to the quantity of sample required, and place in an air tight container

2

PREPARATION OF SAMPLE—OFFICIAL

Pass the entire sample submitted to the chemist thru a 10-mesh sieve before subdividing for analysis Reduce the gross sample by quartering to a quantity sufficient for analytical purposes Transfer the sample to a sieve having circular openings 1/25 inch (1 mm) in diameter and sift breaking the lumps with a pestle Grind the portion remaining on the sieve until all particles pass thru, grinding and sifting as rapidly as possible to avoid loss or gain of moisture during the operation Mix thoroly and preserve in tightly stoppered bottles

3

MECHANICAL ANALYSIS OF BONE AND TANKAGE—OFFICIAL

Transfer 100 g of the original material to a sieve having circular openings 1/50 inch (0 5 mm) in diameter Sift, breaking the lumps by means of a soft rubber pestle if the material has a tendency to cake Weigh the coarse portion remaining on the sieve Determine the fine portion by difference

4

MOISTURE—OFFICIAL

Heat 2 g of the sample prepared as directed under 2 for 5 hours in a water oven at the temperature of boiling H_2O (98–100°) In the case of potash salts $NaNO_3$, and $(NH_4)_2SO_4$ heat at about 130° to constant weight Report the percentage loss in weight as moisture

5

TOTAL PHOSPHORIC ACID

Gravimetric Method—Official

REAGENTS

(a) *Molybdate soln* —Dissolve 100 g of molybdic acid (MoO_3) in a mixture of 144 cc of NH_4OH and 271 cc of H_2O Pour this soln slowly and with constant stirring into a mixture of 489 cc of HNO_3 and 1148 cc of H_2O Keep the final mixture in a warm place for several days or until a portion heated to 40 deposits no yellow precipitate of NH_4 phosphomolybdate Decant the soln from any sediment and preserve in glass stoppered vessels

(b) *Ammonium nitrate soln* —Dissolve 100 g of commercial NH_4NO_3 phosphate free, in H_2O and dilute to 1 liter

(c) *Magnesia mixture* —(1) Dissolve 11 g of MgO in HCl (1+4), avoiding an excess of the acid add a little MgO in excess, boil a few minutes to precipitate Fe, Al, and P_2O_5, and filter To the filtrate add 140 g of NH_4Cl and 130 5 cc of NH_4OH

Volumetric Method—Official

8 REAGENTS

(a) *Molybdate soln* —To 100 cc of molybdate soln, prepared as directed under 5(a), add 5 cc of HNO_3 Filter this soln immediately before using

(b) *Standard sodium or potassium hydroxide soln* —Dilute 323 81 cc of *N* alkali free from carbonates, to 1 liter, 100 cc of the soln should neutralize 32 38 cc of *N* acid, 1 cc = 1 mg or 1% of P_2O_5 on a basis of 0 1 g of substance

(c) *Standard acid soln* —Prepare a soln of HCl or of HNO_3 corresponding to the strength of (b), or to ½ of this strength, and standardize by titration against that soln, using the phenolphthalein indicator

(d) *Phenolphthalein indicator* —Dissolve 1 g of phenolphthalein in 100 cc of alcohol, 95% by volume

9 PREPARATION OF SOLUTION

Treat 2 g of the sample as directed under 6(a), (b), (c) or (d), preferably (a) when these acids are a suitable solvent, and dilute to 200 cc with H_2O

10 DETERMINATION[6]

(a) For percentages up to 5 use an aliquot corresponding to 0 4 g of substance for percentages between 5 and 20 use an aliquot corresponding to 0 2 g of substance, and for percentages above 20 use an aliquot corresponding to 0 1 g of substance Add 5–10 cc of HNO_3, depending on the method of soln (or the equivalent in NH_4NO_3), add NH_4OH until the precipitate that forms dissolves but slowly on stirring vigorously, dilute to 75–100 cc, and adjust to a temp of 25–30° For percentages below 5, add 20–25 cc of the freshly filtered molybdate soln, for percentages between 5 and 20 add 30–35 cc of the molybdate soln, and for percentages greater than 20 add sufficient molybdate soln to insure complete precipitation Place the soln in a shaking or stirring apparatus and shake or stir for 30 min at room temp, decant *at once* thru a filter, and wash the precipitate twice by decantation with 25–30 cc portions of H_2O, agitating thoroly and allowing to settle, then transfer the precipitate to the filter and wash with cold H_2O until the filtrate from 2 fillings of the filter yields a pink color upon the addition of phenolphthalein and 1 drop of the standard alkali Transfer the precipitate and filter to the beaker or precipitating vessel, dissolve the precipitate in a small excess of the standard alkali, add a few drops of phenolphthalein indicator, and titrate with the standard acid

(b) *Not applicable in the presence of sulfates* —Proceed as directed under (a) to the point where the soln is diluted to 75–100 cc Then heat in a water bath to 15–50°, add the molybdate soln at the rate of 75 cc for each decigram of P_2O_5 present, allow the mixture to remain in the bath, stirring occasionally for 30 min, decant *at once* thru a filter, wash, and titrate as directed under (a)

WATER SOLUBLE PHOSPHORIC ACID

Gravimetric Method—Official

11 Place 2 g of the sample* on a 9 cm filter and wash with successive small portions of water, allowing each portion to pass thru before adding more until the filtrate measures about 250 cc If the filtrate is turbid add 1–2 cc of HNO_3 Dilute to a convenient volume, mix well, and proceed as directed under 7

The use of 1 g (instead of 2 g as given here) followed by 1 hour a digestion (see sec 14) was approved as official, first action at the 1930 meeting but this change will not become official until final action has been taken.

Volumetric Method—Official

12 Treat the sample as directed under 11 To an aliquot of the soln corresponding to 0 2 or 0 4 g, add 10 cc of HNO_3, nearly neutralize with NH_4OH dilute to 60 cc, and proceed as directed under 10

CITRATE INSOLUBLE PHOSPHORIC ACID—OFFICIAL

REAGENTS

13 *Ammonium citrate soln* —Prepare according to either of the following methods preferably (2)

(1) Dissolve 370 g of crystallized citric acid in 1500 cc of H_2O, nearly neutralize with strong NH_4OH, cool add strong NH_4OH until exactly neutral to corallin (saturated alcoholic soln) and dilute sufficiently to make the sp gr 1 09 at 20° The volume will be about 2 liters

(2)[1] For every liter of soln required dissolve 172 g of anhydrous or 188 13 g of crystallized citric acid in approximately 700 cc of H_2O, nearly neutralize with strong NH_4OH, cool, measure the volume of the soln or make it up to a convenient volume, taking care to keep the density above 1 09 and make exactly neutral, testing as follows

With a pipet transfer 5 cc of the citrate soln to a test tube (7 × ¾ inches is a convenient size) and dilute to 20 cc with H_2O Add from a dropping bottle 5 drops of a 0 08% soln of phenol red indicator, either an alcoholic soln of the dye or an aqueous soln of its alkali salt being suitable From a buret run in approximately 2 *N* NH_4OH until the color approximates that of a standard buffer soln having a pH of 7 0 contained in a similar test tube and with the same concentration of indicator (This soln may be prepared by mixing 50 cc of 0 2 *M* KH_2PO_4 soln and 29 54 cc of 0 2 *N* NaOH soln and making up to 200 cc[2] Chemicals especially purified for this purpose should be employed and the standard soln finally used should not stand more than a few days unless some method of checking its reaction is available The colorimetric pH standard solns obtainable from chemical supply houses may be used These standards will retain their accuracy for an indefinite period if they are protected from excessive exposure to light and heat) Complete the process by adding the approximately 2 *N* NH_4OH in small quantities and comparing the colors in a comparator[4] From the quantity of NH_4OH soln required to produce in the sample a color that exactly matches that of the standard calculate the quantity required to neutralize the rest of the soln Add this calculated quantity of NH_4OH to the original soln and check its reaction against that of the neutral standard using the technic described above When the colors match dilute the soln to a density of 1 09 at 20

The other reagents and solns used are described under 5 and 8

DETERMINATION

14 (a) *Acidulated samples* —Heat 100 cc of the NH_4 citrate soln to 65° in a 250 cc flask placed in a water bath keeping the flask loosely stoppered to prevent evaporation Keep the level of the water in the bath above that of the citrate soln in the flask When the temp of the citrate soln has reached 65°, drop into it the filter containing the residue from the water soluble P_2O_5 soln under 11, close the flask tightly with a smooth rubber stopper, and shake vigorously until the filter paper is reduced to a pulp relieving the pressure by momentarily removing the stopper Return the flask to the bath and maintain its contents at exactly 65° Shake the flask every 5 min

At the expiration of exactly 30 min * from the time the filter and the residue were introduced, remove the flask from the bath and immediately filter the contents as rapidly as possible thru a quick acting filter Wash with H O at 65° until the volume of the filtrate is about 350 cc, allowing time for thoro draining before adding new portions of H_2O Determine the P_2O_5 in the citrate insoluble residue by one of the following methods (1) Dry the filter and its contents, transfer to a crucible ignite until all organic matter is destroyed, and digest with 10–15 cc of HCl until all phosphate is dissolved, (2) transfer the wet filter with contents to a 200 cc flask, add 30–35 cc of HNO_3 and 5–10 cc of HCl, and boil until all phosphate is dissolved, or (3) treat the filter and its contents as directed under 6 (c) or (d) Dilute the soln to 200 cc, mix well filter thru a dry filter, and proceed as directed under 7 or 10

(b) *Non acidulated samples* —In case a determination of citrate insoluble P_2O_5 is required in non acidulated samples, treat 2 g† of the phosphatic material without previous washing with water as directed under (a) and determine P_2O_5 as directed under 7 or 10 If the substance contains much animal matter (bone, fish, etc), dissolve the residue insoluble in NH_4 citrate by one of the processes described under 6 (c) or (d)

(c) *Precipitated phosphates* [5]—Without washing previously with H_2O treat 1 g of the material as directed under (a)

15 CITRATE SOLUBLE PHOSPHORIC ACID—OFFICIAL

Subtract the sum of the water soluble and citrate insoluble P_2O_5 from the total to obtain the citrate soluble P_2O_5

16 DETECTION OF NITRATES—OFFICIAL

Mix 5 g of the fertilizer with 25 cc of hot H_2O, and filter To 1 volume of this soln add 2 volumes of H_2SO_4 free from HNO_3 and oxides of N, and allow the mixture to cool Add a few drops of a concentrated soln of ferrous sulfate in such a manner that the fluids will not mix If nitrates are present, the junction shows at first a purple, afterwards a brown, color or if only a very minute quantity is present, a reddish color To another portion of the soln add 1 cc of a 1% soln of $NaNO_3$ and test as before to determine whether sufficient H_2SO_4 was added in the first test

ORGANIC AND AMMONIACAL NITROGEN ONLY

Kjeldahl Method—Official

17 REAGENTS

For ordinary work 0 5 N acid is recommended for work in determining very small quantities of N 0 1 N acid is recommended In titrating mineral acids against a soln of NH_4OH use cochineal or methyl red as indicator

(a) *Standard hydrochloric acid* —Determine the absolute strength as follows

PRELIMINARY TEST Place a measured portion of the acid to be standardized in an Erlenmeyer flask and add an excess of $CaCO_3$ to neutralize free acid and a few drops of a 10% soln of K_2CrO_4 as indicator Titrate with 0 1 N $AgNO_3$ soln and note the exact quantity required to precipitate the chlorides

FINAL DETERMINATION To a measured portion of the acid to be standardized add from a buret 1 drop in excess of the required quantity of $AgNO_3$ soln as determined by the preliminary test Heat the mixture to boiling, protect from the light, and

* Digestion for 1 hour (instead of exactly 30 min as here given) was approved as official first action (see footnote under sec 11)

† The use of 1 g (instead of 2 g as here given) was approved as official first action at the 1930 meeting

allow to stand until the precipitate is granular Filter on a Gooch crucible, previously heated to 140–150° and weighed, wash with hot H_2O, testing the filtrate to verify an excess of $AgNO_3$ Dry the AgCl at 140–150°, cool, and weigh

(b) *Standard sulfuric acid* —Determine the strength of the acid by precipitation with $BaCl_2$ soln as follows Dilute a measured quantity of the acid to be standardized to approximately 100 cc, heat to boiling, and add, dropwise a 10% soln of $BaCl_2$ until no further precipitation occurs Continue the boiling for about 5 min allow to stand for 5 hours or longer in a warm place pour the supernatant liquid on a weighed Gooch crucible or an ashless filter, treat the precipitate with 25–30 cc of boiling H_2O, transfer to the filter, and wash with boiling H_2O until the filtrate is free from chlorides Dry, ignite over a Bunsen burner and weigh as $BaSO_4$

(c) *Standard alkali soln* —A 0 1 *N* soln is recommended Accurately determine the strength of this soln by titration against the standard acid prepared as directed under (a) or (b), or proceed as follows Place a weighed quantity of pure anhydrous benzoic acid (Bureau of Standards Sample No 39 is recommended), in a 300 cc flask that has been swept free from CO_2, add 20 cc of alcohol stopper, and let stand until the sample has dissolved, add 3 drops of a 1% soln of phenolphthalein and titrate directly with the alkali soln taking precautions to prevent the entrance of CO_2 into the flask The effect of the alcohol on the end point should be determined by a blank titration It is important that the alkali soln be free from CO_2 Such a soln may be prepared by dissolving 100 g of NaOH in 100 cc of distilled H_2O and allowing the soln to stand in a stoppered flask until the supernatant liquid is clear The required quantity of NaOH is then removed by means of a pipet and quickly diluted to the proper volume with CO_2-free distilled H_2O This should be protected from CO_2 by means of a suitable soda lime guard tube An alkali soln that has been standardized in this way may be used in place of the procedures described under (a) and (b) for the standardization of the hydrochloric and sulfuric acid solns

(d) *Sulfuric acid* —Should contain 93–96% H_2SO_4 and be free from nitrates and $(NH_4)_2SO_4$

(e) *Metallic mercury or mercuric oxide* —Should be prepared in the wet way but not from $Hg(NO_3)_2$

(f) *Copper sulfate* —Crystallized

(g) *Granulated zinc or pumice stone* —Add to the contents of the distillation flask to prevent bumping

(h) *Sulfide, or thiosulfate soln* —Dissolve 40 g of commercial K_2S in 1 liter of H_2O A soln of 40 g of Na_2S or 80 g of $Na_2S_2O_3 \cdot 5H_2O$ in a liter may be used

(i) *Sodium hydroxide soln* —Dissolve approximately 450 g of commercial NaOH free from nitrates in 1 liter of H_2O This soln should have a sp gr of 1 43–1 48

(j) *Cochineal indicator* —Digest 3 g of pulverized cochineal in a mixture of 50 cc of 95% alcohol and 200 cc of H_2O for 1 or 2 days at ordinary temp , agitating frequently, and then filter

(k) *Methyl red indicator* —Dissolve 1 g of methyl red (dimethylaminoazobenzene orthocarboxylic acid) in 50 cc of 95% alcohol dilute to 100 cc with H_2O and filter if necessary

Test reagents before using by a blank determination with sugar The sugar insures partial reduction of any nitrates present

APPARATUS

18

(a) *Kjeldahl flasks for both digestion and distillation* —Total capacity of about 550 cc made of hard, moderately thick and well annealed glass

(b) *Distillation flask* —Use any suitable flask of about 550 cc capacity, fitted with a rubber stopper thru which passes the lower end of a Kjeldahl connecting bulb to prevent NaOH being carried over mechanically during the distillation Use a bulb about 3 cm in diameter, and tubes of the same diameter as the condenser tube, with which the upper end of the bulb tube is connected by means of rubber tubing

19 DETERMINATION

Place 0 7–3 5 g, according to the N content, of the substance to be analyzed in a digestion flask Add approximately 0 7 g of HgO, or its equivalent in metallic Hg, and 20–30 cc of H_2SO_4 (0 1–0 3 g of crystallized $CuSO_4$ may also be used in addition to the Hg, or in many cases, in place of it) Place the flask in an inclined position and heat below the boiling point of the acid until frothing has ceased (A small piece of paraffin may be added to prevent extreme foaming) Increase the heat until the acid boils briskly and digest for a time after the mixture is colorless or nearly so, or until oxidation is complete (The digestion usually requires at least 2 hours)

After cooling, dilute with about 200 cc of H_2O, and add a few pieces of granulated *Zn or pumice stone to prevent bumping* and 25 cc of the K_2S or Na_2S or $Na_2S_2O_3$ soln with shaking (If $Na_2S_2O_3$ is to be used, it should first be mixed with the NaOH so that they may be added together When no Hg or HgO is used the addition of K_2S or Na_2S or $Na_2S_2O_3$ soln is unnecessary) Next add sufficient NaOH soln to make the reaction strongly alkaline (50 cc is usually sufficient), pouring it down the side of the flask so that it does not mix at once with the acid soln Connect the flask to the condenser by means of a Kjeldahl connecting bulb, taking care that the tip of the condenser extends below the surface of the standard acid in the receiver, mix the contents by shaking, and distil until all NH_3 has passed over into a measured quantity of the standard acid (The first 150 cc of the distillate will generally contain all the NH_3) Titrate with standard alkali soln, using the methyl red or cochineal indicator

Gunning Method—Official

20 REAGENTS

Potassium sulfate, or anhydrous sodium sulfate —Pulverized The other reagents and solns used are described under 17

21 APPARATUS

Use the apparatus described under 18

22 DETERMINATION

Place 0 7–3 5 g, according to the N content, of the substance to be analyzed in a digestion flask Add 10 g of powdered K_2SO_4 or anhydrous Na_2SO_4 and 15–25 cc (ordinarily about 20 cc) of concentrated H_2SO_4 (0 1–0 3 g of crystallized $CuSO_4$ may also be added) Conduct the digestion as in the Kjeldahl process, starting with a temp below the boiling point and increasing the heat gradually until frothing ceases Digest for a time after the mixture is colorless or nearly so, or until oxidation is complete (The digestion usually requires at least 2 hours) Complete as directed under 19, but do not add K_2S or Na_2S or $Na_2S_2O_3$ In making the mixture alkaline before distilling add litmus paper or a few drops of phenolphthalein indicator (The pink color given by phenolphthalein indicating an alkaline reaction, is destroyed by a considerable excess of strong fixed alkali)

Kjeldahl Gunning Arnold Method—Official

23 REAGENTS AND APPARATUS

Use the apparatus, reagents, and solns described under 17, 18, and 20

24 DETERMINATION

Place 0 7–3 5 g according to the N content, of the substance to be analyzed in a digestion flask Add 15–18 g of K_2SO_4 or anhydrous Na_2SO_4, 1 g of $CuSO_4$ or approximately 0 7 g of HgO (or its equivalent in metallic Hg) and 25 cc of H_2SO_4 Heat the mixture gently until frothing ceases then boil briskly and continue the digestion for a time after the mixture is colorless or nearly so, or until oxidation is complete (The digestion usually requires at least 2 hours) Cool, add about 200 cc of H_2O, and if HgO or metallic Hg has been used, add also 50 cc of the K_2S or Na_2S or Na S_2O_3 soln, make strongly alkaline with the NaOH, and complete as directed under 19

TOTAL NITROGEN

Kjeldahl Method Modified to Include the Nitrogen of Nitrates—Official

25 REAGENTS

(a) *Zinc dust* —An impalpable powder Granulated Zn or Zn filings are not satisfactory

(b) *Sodium thiosulfate* ($Na_2S_2O_3 \cdot 5H_2O$)

(c) *Commercial salicylic acid*

The other reagents and solns are described under 17

26 APPARATUS

Use the apparatus described under 18

27 DETERMINATION

Place 0 7–3 5 g, according to the N content, of the substance to be analyzed in a Kjeldahl digestion flask (1) Add 30 cc of H_2SO_4 containing 1 g of salicylic acid, shake until thoroly mixed allow to stand for at least 30 min with frequent shaking or until complete soln results and then add 5 g of crystallized $Na_2S_2O_3$ and digest as directed below, or, (2) add to the substance 30 cc of H_2SO_4 containing 2 g of salicylic acid, allow to stand at least 30 min with frequent shaking or until complete soln results and then add gradually 2 g of Zn dust shaking the contents of the flask at the same time and digest as follows

Heat over a low flame until all danger from frothing has passed Then increase the heat until the acid boils briskly and continue the boiling until white fumes no longer escape from the flask (5–10 min) Add approximately 0 7 g of HgO, or its equivalent in Hg, and continue the boiling until the liquid in the flask is colorless, or nearly so If the contents of the flask are likely to become solid before this point is reached, add 10 cc more of H_2SO_4 Complete the determination as directed under 19 Test the reagents by blank determinations

Gunning Method Modified to Include the Nitrogen of Nitrates—Official

28 REAGENTS AND APPARATUS

Use the apparatus, reagents, and solns described under 17, 18, 20, and 25

29 DETERMINATION

Place 0 7–3 5 g according to the N content of the substance to be analyzed in a digestion flask Add 30–35 cc of salicylic acid mixture (1 g of salicylic acid to 30

cc of H_2SO_4), shake until thoroly mixed, and allow to stand, shaking frequently, for at least 30 min , or until complete soln results Add 5 g of Na S_2O_3 and heat the soln for 5 min , cool, add 10 g of K_2SO_4 or anhydrous Na SO_4, heat very gently until foaming ceases, and proceed as directed under 22

AMMONIACAL NITROGEN

30 *Magnesium Oxide Method—Official*

Place 0 7–3 5 g, according to the NH_3 content, of the substance to be analyzed in a distillation flask with about 200 cc of H_2O and 2 g* or more of MgO, free from carbonates Connect the flask with a condenser by means of a Kjeldahl connecting bulb, distil 100 cc of the liquid into a measured quantity of standard acid, and titrate with standard alkali, using cochineal or methyl red indicator

NITRIC AND AMMONIACAL NITROGEN

31 *Reduced Iron Method—Official*

(Applicable only in the absence of cyanamides and urea)

Place 0 7 or 1 g of the sample in a 500 cc flask, add about 30 cc of H_2O and 2–3 g of reduced Fe, and, after allowing the mixture to stand sufficiently long to insure soln of the soluble nitrates and NH_4 salts, add 10 cc of H_2SO_4 (1+1) Shake thoroly, place a long stemmed funnel in the neck of the flask to prevent mechanical loss, and allow to stand until the violence of the reaction has moderated Heat the soln slowly, boil for 5 min , and cool Add about 100 cc of H_2O, a little paraffin, and 7–10 g of MgO, free or nearly free from carbonates Connect the flask by means of a Kjeldahl connecting bulb with a condenser, such as is used in the Kjeldahl method, and boil the mixture nearly to dryness (about 40 min), collect the NH_3 in a measured quantity of standard acid, and titrate with standard alkali soln, using cochineal or methyl red indicator The N obtained represents the nitrates plus the NH_4 salts contained in the sample

In the analysis of nitrate salts proceed as above, but use 25 cc of the nitrate soln equivalent to 0 25 g of the sample, with 5 g of reduced Fe After boiling, add 75 cc of H_2O and an excess of NaOH soln and complete the determination as above

NITRATE NITROGEN

32 *Robertson Method—Official, First Action*[6]

(Applicable in the presence of cyanamides and urea)

(a) Determine the total N as directed under 27 or 29

(b) Weigh out 2 0 g of the fertilizer mixture on a filter, wash with H_2O to nearly 200 cc in a graduated flask, and make up to volume Determine the N in the residue as directed under 19, 22, or 24

(c) Determine the ammoniacal N in 50 cc of the filtrate as directed under 30

(d) Place another 50 cc portion of the filtrate in a 500 cc Kjeldahl flask and add 2 g of ferrous sulfate and 20 cc of H_2SO_4 (sp gr 1 84) (If the total N is over 5%, use 5 g of ferrous sulfate) Digest over a hot flame until all the H_2O is evaporated and white fumes appear and continue the digestion for at least 10 min to drive off the nitrate N If severe bumping occurs, add 10–15 glass beads Add 0 65 g of Hg or its equivalent of HgO and digest until all the organic matter is oxidized Cool, dilute, add the K_2S soln, and complete the determinaton as directed under 24 (A pinch of a

*2 g has been approved as official first action The method as printed in the 2nd Edition of this book read 5 grams

mixture of Zn dust and granular Zn (20-mesh) should be added to each flask before distillation to prevent bumping)

Total N (a)—water insoluble N (b) = water soluble N Water soluble N—the N obtained in (d) = the nitrate N

Ammoniacal N + nitrate N = mineral N Total N − mineral N = organic N

33 *II Jones Modification of the Robertson Method*

(Applicable when a determination of water soluble nitrogen is not needed)

Weigh 0.5 g of the sample into a Kjeldahl flask Add 50 cc of H_2O and rotate gently, then add 2 g of ferrous sulfate and rotate Add 20 cc of H_2SO_4 (sp gr 1.84) Digest over a hot flame When the water is evaporated and white fumes appear add 0.65 g of Hg and complete the digestion as in the regular Kjeldahl method Cool dilute, and distil as usual The total N − the N thus found = the nitrate N

NITROGEN IN NITRATE SALTS

34 *I Ferrous Sulfate Zinc Soda Method—Official*

Place 0.3, 0.4, or 0.7 g of the nitrate salt in a 600–700 cc flask and add 200 cc of H_2O, 5 g of powdered Zn, 1–2 g of ferrous sulfate, and 50 cc of NaOH soln (sp gr 1.34) Connect with the distilling apparatus distil, collect the distillate in the usual way in 0.1 N H_2SO_4 and titrate with standard alkali using cochineal or methyl red indicator

35 *II Devarda Method—Official, First Action*

Place 0.5 g of the nitrate salt in a 600–700 cc flask and add 300 cc of H_2O 3 g of the Devarda alloy[8] and 5 cc of NaOH soln (42% by weight), pouring the latter down the side of the flask so that it does not mix at once with the contents Connect by means of a Davisson[9] or other suitable scrubbing bulb that will prevent the passing over of any portion of the spray with a condenser, the tip of which always extends beneath the surface of the standard acid in the receiving flask Mix the contents of the distilling flask by rotating Heat slowly at first and then at such a rate that the 250 cc of distillate required will pass over in 1 hour Collect the distillate in a measured quantity of standard acid and titrate with standard alkali soln, using cochineal or methyl red indicator

NITROGEN ACTIVITY METHODS

WATER INSOLUBLE ORGANIC NITROGEN SOLUBLE IN NEUTRAL PERMANGANATE—OFFICIAL

36 *Preliminary Test (Determination of Water Insoluble Organic Nitrogen)*

Place 1 or 1.4 g of the material on an 11 cm filter paper and wash with H_2O at room temp until the filtrate measures 250 cc Dry and determine N in the residue as directed under 19 or 22, making a correction for water insoluble N of the filter paper if necessary

37 DETERMINATION

Place the quantity of fertilizer equivalent to 50 mg of water insoluble organic N, as determined under 36, on a moistened 11 cm filter paper and wash with H_2O at room temp until the filtrate measures 250 cc Transfer the insoluble residue with 250 cc of tepid H_2O to a 400 cc Griffin low form beaker Add 1 g of Na_2CO_3, mix and add 100 cc of a 2% soln of $KMnO_4$ Cover with a watch glass and immerse for 30 min in a steam or hot water bath, keeping the liquid in the beaker below that

of the H_2O in the bath Stir twice at intervals of 10 min At the end of 30 min remove from the bath, add immediately 100 cc of cold H_2O, and filter thru a heavy 15 cm folded filter Wash with small quantities of cold H_2O until the filtrate measures about 400 cc Determine N in the residue and filter, as directed under 19 or 22, correcting for the N contained in the filter The N thus obtained is the inactive water insoluble organic N The N obtained under 36—the percentage of N found = the water-insoluble organic N soluble in neutral permanganate

WATER INSOLUBLE ORGANIC NITROGEN DISTILLED FROM ALKALINE PERMANGANATE—OFFICIAL

38 REAGENTS

(a) *Stock soln of potassium permanganate* —Dissolve 50 g of $KMnO_4$ in a liter of H O Dissolve 0 5 g of Na oxalate in 300 cc of H_2O and 10 cc of concentrated H_2SO_4 Heat to 75–80° and titrate with the $KMnO_4$ soln, using a Mohr pipet or an all glass buret to contain the permanganate soln 235 89—by the result of the titration in cc = the concentration of $KMnO_4$ in grams per liter Adjust the concentration to 50 g per liter, protect from the light, and store at a temp above 15°

(b) *Stock soln of sodium hydroxide* —Dissolve 300 g of NaOH in a liter of H_2O Cool before using

(c) *Alkaline permanganate soln* —Mix equal quantities of the stock solns (a) and (b) and add 10 cc of H_2O for each liter of soln that the mixture is calculated to make Use this soln immediately, as it is unstable

39 PREPARATION OF SAMPLE

(a) *Mixed fertilizers* —Place the quantity of material equivalent to 50 mg of water insoluble organic N, as determined under 36, on a filter paper and wash with H_2O at room temp until the filtrate measures 250 cc

When it is found necessary to use 4 g or more of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed

(b) *Raw materials* —Place the quantity of material equivalent to 50 mg of water insoluble organic N, as determined under 36, in a small mortar, add about 2 g of powdered rock phosphate, mix thoroly, transfer to a filter paper, and wash with *water at room temp until the filtrate measures 250 cc If much oil or fat is present,* it is well to wash with ether before extracting with water

40 DETERMINATION[10]

Dry the residue remaining after treatment of the material as described in 39 at a temp not exceeding 80° and transfer from the filter to a 500–600 cc Kjeldahl distillation flask, loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers Add 20 cc of H_2O, 15–20 small glass beads or fragments of pumice stone, a drop of mineral lubricating oil weighing not more than 50 mg, and 100 cc of alkaline permanganate soln Connect with an upright condenser to the lower end of which has been attached a 100 cc graduated cylinder containing standard acid and so arranged as to receive the distillate below the surface of the acid or otherwise trapped so as to prevent loss of NH_3 fumes Digest slowly with a very low flame for 30 min , barely below distillation point, using coarse wire gauze and asbestos paper between the flask and flame Gradually raise the temp and after all danger from frothing has passed, distil 95 cc in 60 min (plus or minus 5 min), controlling the distillation to obtain approximately 24 cc of distillate in each 15 min period Conduct the first part of the distillation

of the H_2O in the bath Stir twice at intervals of 10 min At the end of 30 min remove from the bath, add immediately 100 cc of cold H_2O, and filter thru a heavy 15 cm folded filter Wash with small quantities of cold H_2O until the filtrate measures about 400 cc Determine N in the residue and filter, as directed under 19 or 22, correcting for the N contained in the filter The N thus obtained is the inactive water insoluble organic N The N obtained under 36—the percentage of N found = the water insoluble organic N soluble in neutral permanganate

WATER INSOLUBLE ORGANIC NITROGEN DISTILLED FROM ALKALINE PERMANGANATE—OFFICIAL

38 REAGENTS

(a) *Stock soln of potassium permanganate* —Dissolve 50 g of $KMnO_4$ in a liter of H_2O Dissolve 0 5 g of Na oxalate in 300 cc of H_2O and 10 cc of concentrated $H SO_4$ Heat to 75–80° and titrate with the $KMnO_4$ soln, using a Mohr pipet or an all-glass buret to contain the permanganate soln 235 89—by the result of the titration in cc = the concentration of $KMnO_4$ in grams per liter Adjust the concentration to 50 g per liter, protect from the light, and store at a temp above 15°

(b) *Stock soln of sodium hydroxide* —Dissolve 300 g of NaOH in a liter of H_2O Cool before using

(c) *Alkaline permanganate soln* —Mix equal quantities of the stock solns (a) and (b) and add 10 cc of H_2O for each liter of soln that the mixture is calculated to make Use this soln immediately, as it is unstable

39 PREPARATION OF SAMPLE

(a) *Mixed fertilizers* —Place the quantity of material equivalent to 50 mg of water insoluble organic N, as determined under 36, on a filter paper and wash with H_2O at room temp until the filtrate measures 250 cc

When it is found necessary to use 4 g or more of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed

(b) *Raw materials* —Place the quantity of material equivalent to 50 mg of water insoluble organic N, as determined under 36, in a small mortar, add about 2 g of powdered rock phosphate, mix thoroly, transfer to a filter paper, and wash with water at room temp until the filtrate measures 250 cc If much oil or fat is present, it is well to wash with ether before extracting with water

40 DETERMINATION[19]

Dry the residue remaining after treatment of the material as described in 39 at a temp not exceeding 80° and transfer from the filter to a 500–600 cc Kjeldahl distillation flask, loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers Add 20 cc of H_2O, 15–20 small glass beads or fragments of pumice stone, a drop of mineral lubricating oil weighing not more than 50 mg, and 100 cc of alkaline permanganate soln Connect with an upright condenser to the lower end of which has been attached a 100 cc graduated cylinder containing standard acid and so arranged as to receive the distillate below the surface of the acid or otherwise trapped so as to prevent loss of NH_3 fumes Digest slowly with a very low flame for 30 min , barely below distillation point, using coarse wire gauze and asbestos paper between the flask and flame Gradually raise the temp and, after all danger from frothing has passed, distil 95 cc in 60 min (plus or minus 5 min), controlling the distillation to obtain approximately 24 cc of distillate in each 15 min period Conduct the first part of the distillation

over a bare flame but use wire gauze 10 min before completion to avoid breaking the flask Transfer the distillate to an Erlenmeyer flask or to a beaker and titrate with standard alkali, using cochineal or methyl red indicator When a tendency to froth is noticed lengthen the digestion period, and no trouble will be experienced when the distillation is begun During the digestion gently rotate the flask occasionally, particularly if the material shows a tendency to adhere to the sides

The N thus obtained is the active water insoluble organic N If active water insoluble N is found to be less than 55% of the total water insoluble organic N present it is recommended that a second portion of the sample be prepared as directed under 39(a) Dry the residue below 80°, transfer from the filter to a Kjeldahl flask as directed above and determine the N as directed under 19 or 22 Recalculate the percentage of active water insoluble N on the basis of the quantity of water insoluble N thus found

Previous to digestion with alkaline permanganate, the washed sample may be transferred from the filter to the flask by spreading the filter on a metal disk bent to form a trough that fits the palm of the hand, brushing the larger portion of the material into the flask with a spatula and washing in the remainder with 20 cc of H O from a 20 cc pipet or small wash bottle Do not add more H_2O before the digestion with alkaline permangate, but with this exception proceed as with the transfer of the dried material

POTASH

Method I Lindo-Gladding[11] —Official

41 REAGENTS

(a) *Ammonium chloride soln* —Dissolve 100 g of NH_4Cl in 500 cc of H_2O, add 5–10 g of pulverized K_2PtCl_6 and shake at intervals for 6–8 hours Allow the mixture to settle overnight and filter The residue may be used for the preparation of a fresh supply

(b) *Platinum soln* —A $PtCl_4$ soln containing the equivalent of 1 g of metallic Pt (2 1 g of H_2PtCl_6) in every 10 cc For materials containing less than 15% of K_2O a $PtCl_4$ soln containing 0 2 g of metallic Pt (0 12 g of H_2PtCl_6) in each 10 cc is recommended

(c) *80% alcohol* —Sp gr 0 8593 at 20/4°

42 PREPARATION OF SOLUTION

(a) *Mixed fertilizers* —Place 2 5 g of the sample upon a 12 5 cm filter paper and wash with successive small portions of boiling H_2O into a 200 cc volumetric flask until the filtrate amounts to about 200 cc Add to the hot soln a slight excess of NH_4OH and sufficient saturated NH_4 oxalate soln to precipitate all the lime present, cool, dilute to 250 cc mix, and pass thru a dry filter

(b) *Potash salts muriate and sulfate of potash, sulfate of potash and magnesia, and kainit* —Dissolve 2 5 g and dilute to 250 cc without the addition of NH_4OH and NH_4 oxalate

(c) *Organic compounds* —When it is desired to determine the total amount of K_2O in organic substances, such as cottonseed meal, tobacco stems, etc , saturate 10 g of the sample with H_2SO_4 and ignite in a muffle at a low red heat to destroy organic matter Add a little HCl, warm slightly in order to loosen the mass from the dish, transfer to a 500 cc volumetric flask add NH_4OH and saturated NH_4 oxalate soln, cool dilute to 500 cc, mix pass thru a dry filter and proceed as directed under 43(a)

(d) *Ashes from wood, cotton hulls, etc* —Boil 10 g of the sample with 300 cc of H_2O for 30 min , and add to the hot soln a slight excess of NH_4OH and then sufficient saturated NH_4-oxalate soln to precipitate all the lime present Cool, dilute to 500 cc, mix, and pass thru a dry filter

43 DETERMINATION

(a) *Mixed Fertilizers* —Evaporate a 50 cc aliquot of soln 42 (a) nearly to dryness, add 1 cc of H_2SO_4 (1+1), evaporate to dryness, and ignite to whiteness Maintain a full red heat until the residue is perfectly white Dissolve the residue in hot H_2O using at least 20 cc for each decigram of K_2O present, and add a few drops of HCl and then an excess of the Pt soln Evaporate on a water bath to a thick paste, avoiding exposure to NH_3 Treat the residue with the 80% alcohol Filter on a Gooch crucible and wash the precipitate thoroly with 80% alcohol, both by decantation and on the filter, continuing the washing after the filtrate is colorless Then wash 5 or 6 times with 10 cc portions of the NH_4Cl soln to remove impurities from the precipitate Wash again thoroly with the 80% alcohol and dry the precipitate for 30 min at 100° Weigh and calculate to K O The precipitate should be completely soluble in water

(b) *Muriate of potash* —Acidify 50 cc of the soln prepared according to 42 (b) with a few drops of HCl, add 10 cc of the Pt soln, and evaporate to a thick paste Treat the residue as directed under (a)

(c) *Sulfate of potash, sulfate of potash and magnesia, and kainit* —Acidify 50 cc of the soln prepared according to 42(b) with a few drops of HCl and add 15 cc of the Pt soln Evaporate the mixture and proceed as directed under (a), but use 25 cc portions of the NH_4Cl soln

(d) *Water soluble potash in ashes from wood, cotton hulls, etc* —Prepare the soln according to 42(d) and proceed as directed under (a), paying special attention to *the last sentence*

For the conversion of K_2PtCl_6 to KCl use the factor 0 3065, to K_2SO_4, 0 35843, to K_2O, 0 19376

Method II—Official

(In the presence of soluble sulfates use preferably Method I)

44 REAGENTS

Use the reagents and solns described under 41

45 PREPARATION OF SOLUTION

Prepare the soln as directed under 42, omitting in all cases the addition of NH_4OH and NH_4-oxalate

46 DETERMINATION

Dilute 25 cc of the soln prepared as directed under 45 (50 cc if less than 10% of K O is present) to 150 cc, heat to 100°, and add, drop by drop, with constant stirring, a slight excess of a 10% $BaCl_2$ soln Without filtering add in the same manner an excess of a saturated soln of $Ba(OH)_2$ Filter while hot and wash until the precipitate is free from chlorides Add to the filtrate 1 cc of strong NH_4OH, and then a saturated soln of $(NH_4)_2CO_3$ until the excess of Ba is precipitated Heat, and add, in fine powder, 0 5 g of pure oxalic acid or 0 75 g of NH_4-oxalate Filter, wash free from chlorides, evaporate the filtrate to dryness in a Pt dish, and ignite carefully over the free flame below a red heat until all volatile matter is driven off Digest the

residue with hot water, filter thru a small filter, and dilute the filtrate, if necessary, to provide for each decigram of K_2O at least 20 cc of liquid Acidify with a few drops of HCl and add an excess of the Pt soln Evaporate on a water bath to a thick paste, treat the residue repeatedly with 80% alcohol, decanting thru a weighed Gooch crucible or other form of filter, transfer the precipitate to the filter, and wash thoroly with the 80% alcohol Dry for 30 min at 100° and weigh If there is an appearance of foreign matter in the double salt, wash as directed under 43(a) with several portions of 10 cc each of the NH_4Cl soln

THOMAS OR BASIC SLAG

47 MECHANICAL ANALYSIS—OFFICIAL

Proceed as directed under 3, using 10 g of material

48 PREPARATION OF SAMPLE—OFFICIAL

Proceed as directed under 2

TOTAL PHOSPHORIC ACID

Gravimetric Method—Official

49 PREPARATION OF SOLUTION

Prepare the soln for analysis as directed under 6(b), or in HCl alone In the latter case, measure out the portion for analysis add 3 5 cc of HNO_3 and heat for a few min

50 DETERMINATION

Dehydrate an aliquot (20 cc) of the soln, obtained as directed under 49, by evaporating to dryness on a steam or hot water bath, treat with 5 cc of HCl and 25 cc of hot H_2O digest in order to complete soln and filter off SiO_2 From this point proceed as directed under 7 Before precipitating with magnesia mixture add 5 cc of a 5% Na acetate soln

51 *Volumetric Method—Official*

Prepare the soln as directed under 6(b) and determine the P_2O_5 in an aliquot of this soln as directed under 10, standardizing the solns against a standard phosphate material of approximately the same composition as the sample under examination

CITRIC ACID SOLUBLE PHOSPHORIC ACID

Gravimetric Method—Official

52 PREPARATION OF SOLUTION

Weigh 5 g of the slag prepared as directed under 2, into a 500 cc Wagner flask containing 5 cc of 95% alcohol (The neck of the flask should have a width of at least 22 mm and the graduation marks should be at least 8 cm below the mouth) Make up to the mark with 2% citric acid soln of a temp of 17 5 Fit the flask with a rubber stopper and place at once in a rotary apparatus shaking the flask for 30 min at the rate of 30–40 r p m At the end of this time remove the flask, filter immediately on a dry filter, and analyze the soln at once

53 DETERMINATION

To 50 cc of the clear filtrate in a beaker add 100 cc of molybdate soln prepared as directed under 5(a), and place the beaker in a water bath, when the temp of the

contents reaches 65°, remove and cool to room temp Filter and wash the yellow precipitate of NH_4 phosphomolybdate 4 or 5 times with 1% HNO_3 Dissolve the precipitate in 100 cc of cold 2% NH_4OH, nearly neutralize with HCl, add to the soln drop by drop, with continuous stirring, 15 cc of magnesia mixture prepared as directed under 5(c), and proceed as directed under 7

54 *Volumetric Method—Official*

In an aliquot of the clear soln, prepared as directed under 52, determine the P_2O_5 as directed under 10

SPECIAL METHODS

BORIC ACID

WATER SOLUBLE BORIC ACID[12]—OFFICIAL

55 REAGENTS

(a) *Barium chloride soln* —Dissolve 10 g of $BaCl_2$ $2H_2O$ in H_2O and dilute to 100 cc

(b) *Barium hydroxide* —Powdered

(c) *Standard boric acid soln* —0 1 *N*

(d) *Standard sodium hydroxide soln* —Prepare this soln free from carbonates by first making a saturated soln (100 g of NaOH in 100 cc of H_2O) in order to precipitate any Na_2CO_3 present when the soln is allowed to stand in a vessel from which the CO_2 of the air is excluded Filter thru a hard filter that has been soaked in alcohol, dilute a portion with boiled and cooled H_2O to about 0 1 *N*, and accurately determine the strength of the soln by titration, as described under 56(a), against the standard boric acid

(e) *Hydrochloric acid soln* —(1) Approximately 0 1 *N* and (2) approximately 0 5 *N* soln

(f) *Neutral mannitol (mannite)*

(g) *Methyl red indicator* —Dissolve 0 1 g of methyl red in 50 cc of 95% alcohol, dilute to 1 liter, and filter if necessary

(h) *Phenolphthalein indicator* —Prepare as directed under 8(d)

56 DETERMINATION

(a) *Mineral salts* —Dissolve 5–10 g of the sample in 50–75 cc of hot H_2O, decompose carbonates, if present, with a slight excess of HCl, heat to boiling, and add sufficient 10% $BaCl_2$ soln to precipitate the sulfates, using about 10 cc in excess Next add in small quantities sufficient powdered $Ba(OH)_2$ to make the soln alkaline, avoiding a large excess, boil for about 5 min or until any NH_3 present has been expelled *filter, wash into a 300 cc flask and make acid with HCl using an excess* equivalent to a few cc of 0 1 *N* soln Boil for 15 min to expel CO_2, cool by placing the flask in cold water, and bring to neutrality by first adding 4 or 5 drops of the methyl red indicator and then the standard NaOH soln until the color of the soln changes from pink to yellow If the neutral point has been exceeded, or if there is any doubt as to this restore the pink color by adding a few drops of the approximately 0 1 *N* HCl and change the color to yellow again with the minimum quantity of the standard NaOH soln Add 1–2 g of neutral mannitol and a few tenths of a cc of phenolphthalein indicator note the buret reading, and again titrate the soln with the standard NaOH soln until a pink color develops Add a little more mannitol and if the pink color disappears continue the addition of the standard alkali until it reappears

Repeat until the addition of *mannitol* has no further action on the end point (If the content of boric acid in the soln titrated is low, one addition of mannitol is usually sufficient) From the volume of the standard alkali required in the titration after the addition of the mannitol, corrected for the volume required when running a blank, calculate the quantity of borax in the sample 1 cc of 0 1 *N* NaOH soln = 0 0062 g of boric acid, or 0 0 0 03 g of anhydrous borax

When an acid soln of the sample to be analyzed gives no precipitate upon the addition of a soln of $CaCl_2$ and sufficient NH_4OH to give an alkaline reaction, phosphates and Fe and Al salts are absent, and that portion of the determination which involves treatment with $BaCl_2$ and $Ba(OH)_2$ for the removal of these constituents may be omitted

(b) *Mixed fertilizers and organic compounds* —Weigh 5 g of the sample into a 250 cc beaker, add 50 cc of hot H_2O, cover with a watch glass, digest for 15–20 min on a water bath, filter, and wash into another beaker of the same capacity Heat the filtrate to boiling and add 15 cc of $BaCl_2$ soln followed without undue loss of time by sufficient powdered $Ba(OH)_2$ to give an alkaline reaction as indicated by phenolphthalein, boil for about 5 min gently to prevent frothing over, filter, and wash Or, if preferred make up to the mark in a volumetric flask and take an aliquot Evaporate the filtrate or aliquot to dryness in a Pt or porcelain dish and ignite the residue (preferably in a muffle furnace at a temp just below redness) until the organic matter is completely carbonized Treat the ignited residue with hot H_2O make slightly acid with HCl heat nearly to boiling, make alkaline again with a slight excess of $Ba(OH)_2$, and filter into a 300 cc flask Acidify with HCl (1 + 9), using an excess equivalent to a few cc of a 0 1 *N* soln boil to expel CO_2 and titrate as directed under (a)

If the $Ba(OH)_2$ has been added in slight excess only there is a tendency for the filtrate to become acid during evaporation with a possible loss of borax It is important, therefore, that the soln be kept alkaline by repeated additions of $Ba(OH)_2$, if necessary, until the evaporation is completed

If the filtrate from the $BaCl_2$ $Ba(OH)_2$ precipitate is titrated in this determination before the soluble organic matter is destroyed the end points in the titration will usually be too indefinite to give accurate results The purpose in evaporating the filtrate and igniting the residue, therefore, is to get rid of soluble organic constituents which interfere with the titration When the sample contains a relatively high boric acid content, in excess of 0 5% a smaller sample may be taken and the quantity of organic matter present may then be too small to interfere seriously with the sharpness of the end points during the titration When such is the case, boil the soln after the addition of the $Ba(OH)_2$ until any NH_3 present has been expelled Omit evaporating the filtrate from the $BaCl_2$ $Ba(OH)_2$ precipitate Add to the filtrate an excess of HCl equivalent to a few cc of a 0 1 *N* soln, boil to expel CO_2, and titrate as directed under the determination of mineral salts

ACID SOLUBLE BORIC ACID[13]—OFFICIAL

57 REAGENTS

(a) *Phosphoric acid* (H_3PO_4)—85%

(b) *Methyl alcohol* —At least 95%

(c) *Hydrochloric acid* —Approximately 1 *N*

The other reagents and solns used are described under 55

58 APPARATUS

The apparatus[14] consists of two 200 cc round bottomed flasks, a Liebig condenser, and a 200 cc Erlenmeyer receiving flask One of the 200 cc round bottomed flasks

No 2, is supplied with a doubly perforated rubber stopper, thru one hole of which passes a glass tube running to the bottom of the flask, thru the other hole is a short tube leading to the condenser The other 200 cc round bottomed flask, No 1, is fitted with a perforated rubber stopper and a short bent tube connected by rubber tubing with the long tube in flask No 2 The whole apparatus is supported by clamps and rings on two stands

59 DETERMINATION

If the material to be examined is a mixed fertilizer or probably contains less than 2% of anhydrous borax, weigh 5 g into flask No 2 If the material is a chemical containing much more than 2% of borax, use 2 g Then add 5 cc of the 85% H_3PO_4 and 20 cc of methyl alcohol and connect the flask with the condenser Add 100 cc of methyl alcohol to flask No 1, which is set in a water bath and connected with flask No 2 Place the receiving flask in position at the end of the condenser and apply sufficient heat to the water bath to keep a steady flow of bubbles of methyl alcohol passing thru flask No 2 Also apply some heat to flask No 2 to keep the volume at about 25 cc Continue the distillation until 100 cc of distillate is obtained, which should require about 30 min When distillation is complete, add 2 or 3 drops of phenolphthalein indicator to the distillate and 5–10 cc of 0 1 *N* NaOH, or enough to give it a permanent pink color Stopper the flask, shake well and connect at once with a condenser by means of a Hopkins or similar bulb, distil off the alcohol, and save for another determination Use a water bath, not a gas burner for this purpose Transfer the residue, which should be not less than 10 cc, to a Pt or porcelain dish, using as little H_2O as possible, and evaporate to dryness on a steam or water bath When dry ignite below redness Then acidify with a few drops of approximately 1 *N* HCl, add 20–25 cc of H_2O, and warm for 1–2 min on a steam bath Filter into a small flask, thoroly wash, dilute to about 50–75 cc, attach to an air cooled condenser, and boil gently for a few min to remove CO_2 Add 3 or 4 drops of the methyl red indicator and then the 0 1 *N* NaOH until the red color just disappears Add about 1 g of mannitol, or less if but a small amount of boron is present (At this point, if boric acid is present, the soln will take on a pinkish color, the depth of color depends on the quantity present 0 01 or 0 02% usually being sufficient to give the color if the soln has been carefully neutralized with the NaOH soln) Then add 2 or 3 drops of phenolphthalein indicator and titrate the soln with the 0 1 *N* NaOH A blank should be run with the reagents but if the NaOH is free from CO_2 the blank should not be more than 0 2 cc Calculate to boric acid or anhydrous borax as directed under 56(a)

CHLORINE[11]—OFFICIAL FIRST ACTION

60 REAGENTS

(a) *Standard silver nitrate soln* —Dissolve about 5 g of pure recrystallized $AgNO_3$ in H_2O and dilute to 1 liter Standardize against pure, dry NaCl and adjust so that 1 cc of the soln is equivalent to 0 001 g of Cl

(b) *Potassium chromate indicator* —Dissolve 5 g of K_2CrO_4 in 100 cc of H_2O

61 DETERMINATION

Place 2 5 g of the sample on an 11 cm filter paper and wash with successive portions of boiling H_2O until the washings amount to nearly 250 cc, collecting the filtrate in a 250 cc volumetric flask Cool dilute to the mark with H_2O, and mix well Pipet 50 cc into a 150 cc beaker, add 1 cc of the K_2CrO_4 indicator, and titrate with the standard $AgNO_3$ soln until the color produced by Ag_2CrO_4 appears as a permanent red

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III SEWAGE*

* See note at bottom of p xv

IV AGRICULTURAL LIMING MATERIALS[1]

1 DIRECTIONS FOR SAMPLING—TENTATIVE

Take a sample that is representative of the lot or shipment and that does not contain a disproportionate quantity of the surface or of any modified or damaged zone in the following manner

(a) *Burnt, or lump lime, in bulk* —Collect a composite sample of not less than 10 shovelfuls per car, with proportionate quantities from smaller lots, taking each shovelful from a different part of the lot or shipment Crush immediately to pass a circular opening 1 inch in diameter, mix thoroly and rapidly, quarter down to a 5 lb sample, and place in a properly labeled, dry, air tight container

(b) *Burnt, or lump lime in barrels* —Select at random 5 barrels from each lot or shipment of 20 tons or less and 1 additional barrel for each additional 5 tons Take not less than 10 lbs from each barrel selected and treat as directed under (a)

(c) *Hydrated lime and ground burnt lime, in bags* —Select 10 bags from different parts of each lot or shipment of 20 tons or less and 1 additional bag for each additional 5 tons From each of the bags sampled withdraw a core from top to bottom by means of a sampling tube, mix these portions thoroly and rapidly on heavy sized paper or oilcloth, quarter down to a 2 lb sample, and place in a properly labeled, dry, airtight container

(d) *Ground limestone and ground marl, in bags* —Proceed as directed under (c)

(e) *Ground limestone, ground burnt lime, and ground marl, in bulk* —By means of a slotted sampling tube, withdraw samples to full sampler depth from 10 points in the lot or shipment mix thoroly and rapidly on heavy sized paper or oilcloth, quarter down to a 2 lb sample, and place in a properly labeled, dry, air tight container

2 PREPARATION OF SAMPLE—TENTATIVE

Grind the sample in a porcelain mortar or porcelain ball mill to pass a 60 mesh sieve, mix thoroly and preserve in an air tight container

NEUTRALIZING VALUE—TENTATIVE

3 REAGENTS

(a) *Sodium hydroxide soln* —0 25 *N* Prepare free from carbonates and store in a bottle provided with a siphon tube and with guard tubes containing soda lime, or other suitable device, to prevent absorption of CO_2 from the air

(b) *Nitric acid* —0 5 *N* Standardize against (a), using phenolphthalein indicator

(c) *Phenolphthalein indicator* —Prepare as directed under II, 8(d)

4 DETERMINATION

Place 0 5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 2, in a 150 cc beaker, add 50 cc of the 0 5 *N* HNO_3, cover the beaker with a watch glass, and boil for 5 min Cool and titrate the excess of acid with the 0 25 *N* NaOH soln, using phenolphthalein indicator Calculate and report the results as percentage of CaO in burnt and hydrated lime and as percentage of $CaCO_3$ equivalent for limestones and marls

CAUSTIC VALUE—OFFICIAL

5 APPARATUS

In this apparatus *A* is a 500 cc Erlenmeyer flask of Pyrex glass, and *F* is a filter cone packed nearly full with cotton, which is covered to a depth of from 2 to 3 mm

with lightly compacted macerated filter paper The filter cone is connected with the syphon tube *B* by means of thick walled rubber tubing The receiving flasks *m* and *n* are calibrated to deliver 50 and 100 cc respectively *S* is a suction flask

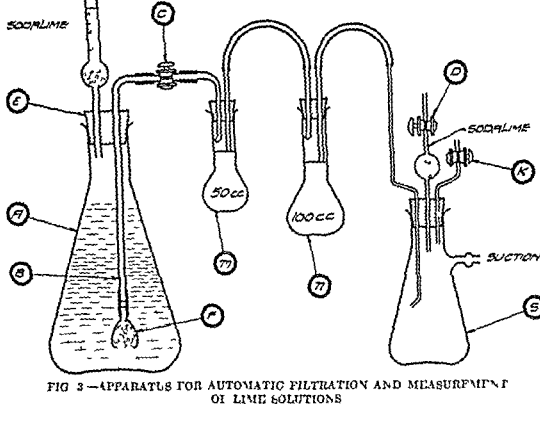


FIG 3 —APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS

6 DETERMINATION

Grind the lime sample to pass a 100 mesh sieve mix thoroly by rolling and place in a sample container (Prepare the sample in an atmosphere of minimum moisture and CO_2 content and protect it from the breath of the operator) Transfer a portion of the sample to a weighing bottle By means of a polished, narrow pointed spatula that has been calibrated to hold approximately 1 5 g withdraw the charge to be used and determine its exact weight by difference Introduce the charge directly into the dry flask (1) provided with a tightly fitting rubber stopper

Prepare a sugar soln *immediately before use* by placing 30 g of granulated sugar in a measuring flask calibrated to deliver 500 cc Dissolve the sugar with cold CO_2-free H_2O and make up to the mark *Hold both* the Erlenmeyer flask containing the charge and the flask containing the sugar soln in a slightly inclined position insert the neck of the sugar soln flask a short distance into the Erlenmeyer flask and carefully transfer the sugar soln while simultaneously and synchronously agitating both flasks by a rotary motion to prevent granulation of the lime Stopper the Erlenmeyer flask securely, agitate, and add if desired a quantity of clean dry beads Effect complete soln of uncoated caustic lime by six 1 min agitations at intervals of 2 or 3 min Crush any undisintegrated particles of the sample by careful twisting of the stopper after inverting the flask to trap them in the space between the stopper and the neck of the flask Allow 1 5 min further contact between the lime and the sugar soln, and then filter

Connect the filter cone F with the syphon B and close stopcock D Connect the receiving flasks, apply suction, and quickly connect the Erlenmeyer flask A containing the lime soln with stopper E Open stopcock C and filter 25–50 cc of the soln Close C and open D to release suction Remove m and replace with another dry flask of the same kind Close D, open C, and continue the filtration until both m and n have been filled at least to the marks To disconnect the system, close stopcock C and press the outlet of flask m down gently and then the outlet of flask n to remove any excess of liquid above the marks Permit the intermediate connection to empty, and then open stopcock D and remove m and n Titrate the first 50 cc, or pilot aliquot, of the filtered soln with 0 5 N HCl, using phenolphthalein indicator Run twice the volume of the 0 5 N acid required for this titration into a covered 200 cc beaker, add the second, or 100 cc aliquot, of the filtered soln to this acid and phenolphthalein indicator, and complete the titration

Calculate the caustic value of the sample by means of the formula

$$V = \frac{7A}{W}, \text{ in which}$$

V = percentage of active CaO,
A = cc of 0 5 N acid used per 100 cc of lime soln,
W = weight of charge

7 CARBON DIOXIDE—TENTATIVE

Proceed as directed under I, 6, using 5 g of burnt or hydrated lime (1 g of ground limestone or ground marl), prepared as directed under 2 Calculate and report the result as percentage of $CaCO_3$

8 TOTAL CALCIUM OXIDE—TENTATIVE

Place 1 g of burnt or hydrated lime (2 g of ground limestone or ground marl), prepared as directed under 2, in a hard glass beaker of 250 cc capacity, add 25 cc of H_2O 10 cc of HCl, and a few drops of HNO_3, boil for 10 min and evaporate to dryness Separate and remove the insoluble matter, SiO_2 and Fe and Al oxides as directed under I, 16 and 17 Determine CaO as directed under I, 18

9 TOTAL MAGNESIUM OXIDE—TENTATIVE

Proceed as directed under I, 19 or 21, using the combined filtrate and washings from the CaO determination under 8

10 MECHANICAL ANALYSIS OF GROUND LIMESTONE—TENTATIVE

Transfer 100 g of the original material to a set of 10 , 20 , 40 , 60 80 , and 100 mesh standardized sieves complying with the specifications of the Bureau of Standards Sift, shaking for 5 min on the 80 and 100 mesh sieves and breaking lumps by means of a soft rubber pestle if the material has a tendency to cake Weigh the material that is retained on each sieve and that which passes the 100 mesh sieve and express as percentages of the total weight

SELECTED REFERENCES

[1] J Assoc Official Agr Chem 7, 252 (1924), U S Bur Standards Circs 96, 118 143, 150, 153, Am Soc Test Materials Tentative Standards 1923 p 277
[2] Ind Eng Chem , 20, 312 (1928), J Assoc Official Agr Chem , 11, 153 (1928)

V AGRICULTURAL DUST*

* See note at bottom of p xv

VI INSECTICIDES AND FUNGICIDES

GENERAL METHODS

1 **PREPARATION OF SAMPLE—OFFICIAL**

Thoroughly mix all samples before analysis. Make water soluble As determinations on samples as received, without further pulverization or drying. In the case of liquids such as NaCN or KCN weigh large quantities in weighing bottles and analyze aliquots of the aqueous solns.

2 **MOISTURE—OFFICIAL**

(Applicable to Paris green, London purple, powdered lead arsenate, calcium arsenate, magnesium arsenate, zinc arsenite, and powdered Bordeaux mixture.)

Dry 2 g to constant weight at 105–110° and report the loss in weight as moisture.

TOTAL ARSENIC

Method I (By Cuprous Chloride Distillation) Official

(Applicable except in the presence of nitrates to the determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

3 **REAGENTS**

(a) *Standard arsenious oxide soln.*—Dissolve 2 g of pure As_2O_3 in a beaker by heating with about 150–200 cc of H_2O containing 10 cc of H_2SO_4, cool, transfer to a 500 cc volumetric flask, and dilute to the mark.

(b) *Standard iodine soln.*—Prepare an approximately 0.05 N soln as follows. Mix intimately 6.35 g of pure I with twice this weight of pure KI. Dissolve in a small quantity of water, filter, and dilute the filtrate to 1 liter in a volumetric flask. Standardize against (a) as follows: Pipet 50 cc of the As_2O_3 soln into an Erlenmeyer flask, dilute to the same volume as that of the aliquot used for the titration in the actual determination, neutralize with NaHCO3, add 1–2 g in excess, and add the standard I soln from a buret, shaking the flask continuously until the yellow color disappears. Add from the soln. Then add 2 cc of the starch indicator and continue adding the I soln dropwise until a permanent blue color is obtained. Calculate the value of the standard I soln in terms of As_2O_3 and As_2O_5. For the conversion of As_2O_3 to As_2O_5 multiply by 1.0152. Occasionally restandardize the I again with the standard As_2O_3 soln.

(c) *Standard bromate soln.*—Dissolve 1.2–1.3 g of $NaBrO_3$ in H_2O and dilute to 1 liter. One cc of this soln is approximately equal to 0.003 g of As_2O_3. To standardize transfer 25 cc aliquots of the standard As_2O_3 soln (a) to 400 cc Erlenmeyer flasks, add 15 cc of HCl, dilute to 100 cc, heat to 90°, and titrate with the bromate soln, using dropping of the methyl orange indicator (d). Do not add the indicator until near the end of the titration and agitate the liquid continuously in order to avoid local excess of the bromate soln. Add the bromate soln very slowly when approaching the end of the titration where the permanent change from red to colorless.

(d) *Methyl orange indicator.*—Dissolve 0.01 g of NaOH in H_2O and dilute to 1 liter.

(e) *Starch indicator.*—Mix about 2 g of finely powdered pure potato starch with cold H_2O to a thin paste, add about 200 cc of boiling H_2O, stir, cool rapidly, and dilute to 1 liter. Preserve with 5 mg of HgI_2 per liter.

(f) *Methyl orange indicator*—Dissolve 1 g of methyl orange in H_2O and dilute to 1 liter

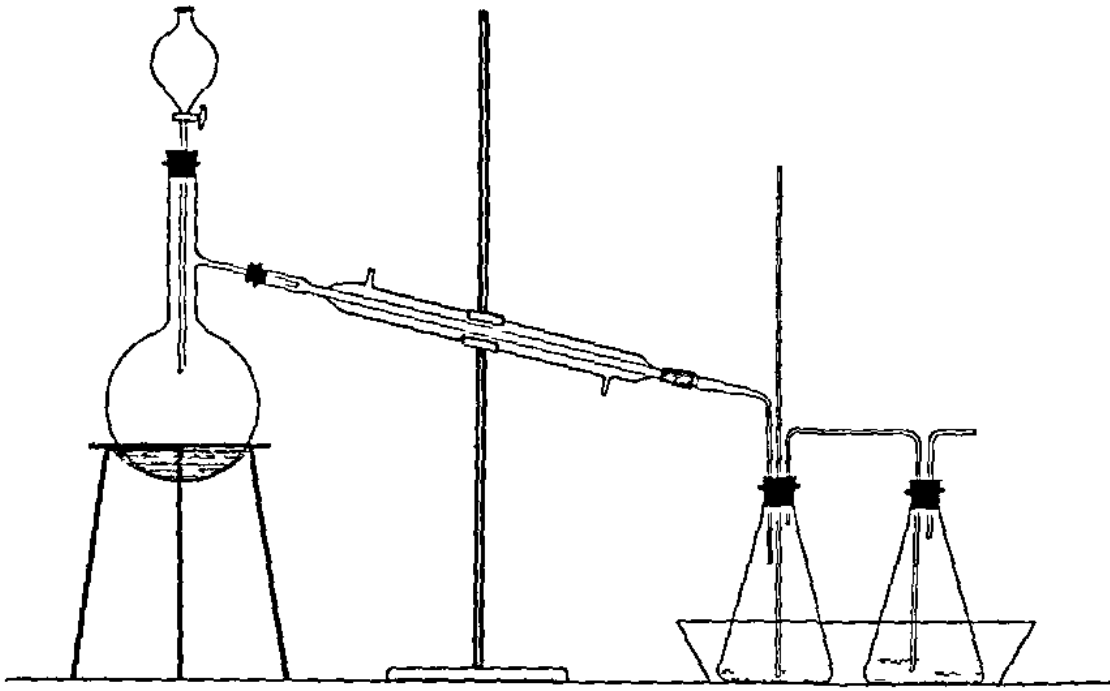


FIG 4—APPARATUS FOR DISTILLATION OF ARSENIOUS CHLORIDE

4 APPARATUS

The apparatus used is shown in Fig 4 The distillation flask is of 500 cc capacity and rests on a metal gauze that fits over a circular hole in a heavy sheet of asbestos board which in turn extends out far enough to protect the sides of the flask from the direct flame of the burner The first receiving flask is of 500 cc capacity and contains 40 cc of H_2O, the second is also of 500 cc capacity and contains 100 cc of H_2O The volume in the first flask should not exceed 40 cc otherwise there may be separated a compound of As that can not readily be redissolved without danger of loss of $AsCl_3$ Keep both flasks cool by placing them in a pan thru which H O circulates or which contains H_2O and pieces of ice

5 DETERMINATION

Weigh a quantity of the sample containing not more than 0 1 g of As and wash into the distillation flask by means of 100 cc of HCl Add 5 g of Cu_2Cl_2 and distil When the volume in the distillation flask is reduced to about 40 cc add 50 cc more of HCl by means of the dropping funnel and continue the distillation, repeating the additions of 50 cc portions of HCl until 200 cc of the acid distillate has passed over Wash down the condenser and all connecting tubes carefully, transfer these washings and the contents of the Erlenmeyer flasks to a liter volumetric flask, dilute to the mark, and mix thoroly Titrate the distillate by one of the following procedures

(a) Pipet a 200 cc aliquot into an Erlenmeyer flask and nearly neutralize with the NaOH soln using a few drops of phenolphthalein indicator [II, 8(d)] and keeping the soln well cooled If the neutral point is passed, add HCl until again slightly acid Neutralize with $NaHCO_3$, add 4–5 g in excess, and add the standard I soln from a buret shaking the flask continuously until the yellow color disappears slowly from

he soln Then add 5 cc of the starch indicator and continue adding the I soln dropwise until a permanent blue color is obtained

(b)[3] Pipet a 200 cc aliquot into an Erlenmeyer flask and titrate with the standard bromate soln as directed under 3(c) beginning with "heat to 90°."

From the number of cc of standard soln used calculate the percentage of As in the sample Report as As_2O_3 or As_2O_5, according to whether the As is present in the trivalent or pentavalent form If the condition of the arsenic is unkown report as As

Method II (By Hydrazine Sulfate Distillation[4])—Official

(Nitrates do not interfere in this method Applicable to the determination of total arsenic in Paris green lead arsenate, calcium arsenate zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals)

REAGENTS

6

Hydrazine sulfate sodium bromide soln —Dissolve 20 g of hydrazine sulfate and 20 g of NaBr in 1 liter of HCl (1 + 1)

The other reagents and solns used are described under 3

APPARATUS

7

The apparatus and manner of connecting are described under 4 (Fig 1)

DETERMINATION

8

Weigh a quantity of the sample containing not more than 0 4 g of As and transfer to the distilling flask Add 50 cc of the hydrazine sulfate NaBr soln close the flask with the stopper that carries the funnel tube and connect the side tube with the condenser Boil for 2 3 min add 100 cc of HCl by means of the dropping funnel and distil until the volume in the distilling flask is reduced to about 40 cc add 50 cc more of HCl and continue the distillation until the contents of the flask are again reduced to about 40 cc Wash down the condenser transfer the contents of the receiving flask to a liter volumetric flask dilute to volume and mix thoroly Titrate the distillate by one of the following procedures

(a) Proceed as directed under 5(a) or

(b) Pipet a 200 cc aliquot into an Erlenmeyer flask add 10 cc of HCl and titrate with the standard bromate soln as described under 3(c) beginning with "heat to 90°."

From the number of cc of standard soln used calculate the percentage of As present in the sample Report as As_2O_3 or As_2O_5 according to whether the As is present in the trivalent or pentavalent form If the condition of the arsenic is unknown report as As

Method III[5] Tentative

(Applicable in presence of [illegible] sulfites thiosulfates or large quantities of sulfur)

REAGENTS

9

[illegible] Approximately 0.0[illegible] N soln [illegible] [illegible] H_2O in H_2O [illegible]

[illegible] described under 3

The other reagents [illegible]

10 APPARATUS

The apparatus and manner of connecting are described under 4 (Fig 4)

11 DETERMINATION

Weigh 2 g of the sample and transfer to the distilling flask Add a soln of 5–8 g of Cu_2Cl_2 in 100 cc of HCl and shake to bring the sample completely in contact with the acid soln and to expel H_2S When the reaction has ceased, close the flask, connect with the condenser, and distil as directed under 5 until 200 cc of the acid distillate has passed over Make the distillate to volume in a liter flask, mix thoroly, and transfer a 200 cc aliquot to a 400 cc Pyrex beaker or porcelain casserole Add 10 cc of HNO_3 and 5 cc of H_2SO_4 evaporate to a sirupy consistency on a steam bath, and then heat on a hot plate until the white fumes of H_2SO_4 appear Cool, and wash into a 500 cc Erlenmeyer flask If the quantity of H_2SO_4 is appreciably lessened by fuming, add sufficient to make the total quantity of H_2SO_4 approximately 5 cc Dilute to 100–150 cc, add 1 5 g of KI, and boil until the volume is reduced to about 40 cc Cool the soln under running H_2O, dilute to 100–150 cc, and add the $Na_2S_2O_3$ soln, 9, dropwise until the I color is just removed Nearly neutralize the H_2SO_4 with the NaOH soln, 3(d), finish the neutralization with $NaHCO_3$ add 4–5 g in excess, and titrate with the standard I soln as directed under 3(b) From the number of cc of standard soln used calculate the percentage of As in the sample Report as As_2O_3 or As_2O_5 according to whether the As is present in the trivalent or pentavalent form If the condition of the arsenic is unknown, report as As

WATER SOLUBLE ARSENIC—OFFICIAL

(Applicable to the determination of water soluble arsenic in lead arsenate, calcium arsenate, zinc arsenite magnesium arsenate, and Bordeaux mixture with arsenicals)

12 REAGENTS

The reagents and solns used are described under 3 and 9

13 DETERMINATION

To 2 g of the original sample if a powder, or 4 g if a paste in a liter Florence flask, add 1 liter of recently boiled H_2O that has been cooled to 32 Stopper the flask and place in a water bath kept at 32° by means of a thermostat Digest for 24 hours, shaking hourly for 8 hours during this period Filter thru a dry filter, transfer 250–500 cc of the *clear* filtrate to an Erlenmeyer flask, add 3 cc of H_2SO_4 and evaporate on a hot plate When the volume reaches approximately 100 cc add 1 g of KI, and continue the boiling until the volume is about 40 cc Cool, dilute to about 200 cc, *and add the* $Na_2S_2O_3$ *soln 9, dropwise until the I color is exactly removed* (Avoid the use of starch indicator at this point) Neutralize with $NaHCO_3$, add 4–5 g in excess titrate with the standard I soln until the yellow color disappears slowly, add 5 cc of the starch indicator, and continue the titration to a permanent blue color Make correction for the quantity of standard I soln necessary to produce the same color using the same reagents and volume From the number of cc of standard I soln used calculate the percentage of water soluble As in the sample

GENERAL PROCEDURE FOR THE ANALYSIS OF PRODUCTS CONTAINING ARSENIC ANTIMONY, LEAD, COPPER ZINC, IRON, CALCIUM, MAGNESIUM, ETC

(Applicable to such preparations as Bordeaux lead arsenate, Bordeaux zinc arsenite Bordeaux Paris green and Bordeaux calcium arsenate)

LEAD OXIDE—OFFICIAL

REAGENTS

14 (a) *Acidified alcohol* —Mix by volume H_2O, 100 parts 95% alcohol 200 parts, and H_2SO_4 3 parts

(b) *Hydrochloric acid* —Approximately 2 *N* Dilute 166 cc of HCl to 1 liter

(c) *Hydrochloric acid* —Approximately 0 5 *N* Dilute 250 cc of (b) to 1 liter

DETERMINATION

15 Weigh 1 g of the powdered sample and transfer to a beaker Add 5 cc of HBr (approximately 1 38 sp gr) and 15 cc of HCl, and evaporate to dryness to remove As repeat the treatment, add 20 cc more of the HCl, and again evaporate to dryness Add to the residue 25 cc of the 2 *N* HCl, heat to boiling, filter immediately to remove SiO_2, and wash with hot H_2O to a volume of 125 cc Take care to see that all $PbCl_2$ is in soln before filtering, if it will not dissolve completely in 25 cc of the 2 *N* acid, add 25 cc additional and dilute the filtrate to 250 cc volume Pa s in H_2S until the precipitation is complete Filter, and wash the precipitate thoroly with the 0 5 *N* HCl saturated with H S Save the filtrate and washings for the determination of zinc Transfer the filter paper containing the sulfides of Pb and Cu to a 400 cc Pyrex beaker and completely oxidize all organic matter by heating on a steam bath with 4 cc of H_2SO_4 and about 20 cc of fuming HNO_3 in a covered beaker, evaporate on the steam bath and then completely remove HNO_3 by heating on a hot plate until copious evolution of the white fumes of H_2SO_4 occurs, cool add 2–3 cc of H_2O, and again heat to fuming, Cool, add 50 cc of H_2O and 100 cc of 95% alcohol, and let stand several hours (preferably overnight) Filter thru a Gooch crucible, previously washed with H_2O, with the acidified alcohol, and with 95% alcohol, and then dried at 200° Wash the precipitate of $PbSO_4$ in the crucible about 10 times with the acidified alcohol, and then with 95% alcohol, to remove H_2SO_4 Dry at 200 to constant weight, keeping the crucible covered to prevent loss from spattering From the weight of $PbSO_4$ calculate the percentage of PbO in the sample using the factor 0 7360

COPPER[3]

Electrolytic Method—Official

16 Evaporate the filtrate and washings from the $PbSO_4$ precipitation, 15, to fuming add a few cc of fuming HNO_3 to destroy organic matter and continue the evaporation until about 3 cc remains Take up with about 100 cc of H_2O add 1 cc of HNO_3 and filter, if necessary Wash into a weighed 150 cc Pt dish and electrolyze using a rotating anode and a current of about 3 amperes (In lieu of the Pt dish a 150 cc beaker and a weighed gauze cathode may be used) After all the Cu has been deposited (requiring about 30 min) and while the current is still flowing wash the deposit with H_2O by siphoning Then interrupt the current, rinse the cathode with alcohol, dry for a few moments in an oven, and weigh Calculate the percentage of Cu in the sample

Thiosulfate Titration Method—Official

17 Proceed as directed under 16 to the point at which the filtrate and washings from the $PbSO_4$ precipitation have been treated with fuming HNO_3 and evaporated to a volume of about 3 cc Take up in about 50 cc of H_2O add NH_4OH in excess and boil until the excess of NH_3 is expelled as shown by a change of color in the liquid and a partial precipitation Then add 3–4 cc of acetic acid (50%) boil 1–2 min cool add 10 cc of a 30% KI soln and titrate with standard thiosulfate soln

(XXXIV, 40) until the brown color becomes faint Then add starch indicator, 3(e), and continue the titration cautiously until the blue color due to free I has entirely vanished From the number of cc of standard thiosulfate soln used calculate the percentage of Cu in the sample

18

ZINC OXIDE[4]—OFFICIAL

REAGENT

Mercury thiocyanate soln —Dissolve 27 g of $HgCl_2$ and 30 g of NH_4SCN in H_2O and dilute to 1 liter

19

DETERMINATION

Concentrate the filtrate and washings from the sulfide precipitation, under 15, by gentle boiling to about 50 cc and continue the evaporation on a steam bath to dryness Dissolve the residue in 100 cc of H_2O containing 5 cc of HCl, and add 35–40 cc of the Hg thiocyanate reagent with vigorous stirring Allow to stand at least an hour with occasional stirring Filter thru a weighed Gooch crucible wash with H_2O containing 20 cc of the Hg thiocyanate reagent per liter, and dry to constant weight at 105° From this weight calculate the percentage of ZnO in the sample, using the factor 0 16332

Some Fe is usually present and during the Zn determination should be in the ferrous condition In making the sulfide precipitation the H_2S should be passed into the soln for a sufficient length of time to reduce the Fe as well as to precipitate the Cu and Pb The Zn Hg thiocyanate precipitate normally is white, and the occluded ferric thiocyanate should not give it more than a faint pink color

20

PARIS GREEN

MOISTURE—OFFICIAL

Proceed as directed under 2

21

TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8

TOTAL ARSENIOUS OXIDE

(The following methods determine only the As present in the trivalent form (As_2O_3) They also determine any Sb that may be present in the trivalent form (Sb_2O_3) Ferrous and cuprous salts vitiate the results)

22

Method I[7]*—Official*

REAGENTS

Ammonium chloride soln —Dissolve 250 g of NH_4Cl in H_2O and dilute to 1 liter

The other reagents and solns are described under 3

23

DETERMINATION

Weigh 0 3 g of the sample and wash into an Erlenmeyer flask with 10–15 cc of HCl (1+4) or 10–15 cc of H_2SO_4 (1+4) followed by about 100 cc of H_2O and heat on a steam bath only so long as is necessary to complete soln at a temp not exceeding 90° (If H_2SO_4 is used the soln may be heated to boiling) Cool neutralize with $NaHCO_3$ add 4–5 g in excess and then add sufficient NH_4Cl soln to dissolve the precipitated Cu Dilute somewhat and titrate as directed under 3(b) Make correction for the quantity of I soln necessary to produce a blue color with starch in the

presence of Cu (using an equivalent weight of $CuSO_4$) From the corrected number of cc of standard I soln used calculate the percentage of As_2O_3 in the sample

Method II[3]*—Official*

24 REAGENTS

The reagents and solns used are described under 3

25 DETERMINATION

Weigh 1 5 g of the sample and wash into a 200 cc volumetric flask with 100 cc of HCl (1+4) heating to a maximum of 90°, if necessary, to secure complete soln of the sample Cool and make to volume

(a) Transfer a 50 cc aliquot to a 500 cc Erlenmeyer flask, add 10 cc of HCl heat to 90°, and titrate with the standard bromate soln as directed under 3(c), beginning with "titrate with the bromate soln " Or,

(b) Proceed as directed under (a) with the exception that the titration is made without heating the soln

From the number of cc of bromate soln used calculate the percentage of As_2O_3 in the sample

SODIUM ACETATE SOLUBLE ARSENIOUS OXIDE—TENTATIVE

26 REAGENTS

(a) *Sodium acetate soln* —Prepare a soln containing 12 5 g of the crystallized salt ($CH_3COONa \cdot 3H_2O$) in each 25 cc

The other reagents and solns used are described under 3

27 DETERMINATION

Place 1 g of the sample in a 100 cc volumetric flask and boil for 5 min with 2 cc of the Na acetate soln Dilute to the mark shake, and pass thru a dry filter paper Titrate an aliquot of this filtrate as directed under 3(b) Calculate the quantity of As_2O_3 present and express the results as percentage of Na acetate soluble As_2O_3

WATER SOLUBLE ARSENIOUS OXIDE—OFFICIAL

28 REAGENTS

The reagents and solns used are described under 3

29 DETERMINATION

To 1 g of the sample in a liter Florence flask add 1 liter of recently boiled H_2O that has been cooled to 32° Stopper the flask and place in a water bath kept at 32° by means of a thermostat Digest for 24 hours, shaking hourly for 8 hours during this period Filter thru a dry filter and transfer 200 cc of the filtrate to an Erlenmeyer flask, add 4–5 g of $NaHCO_3$ and titrate with the standard I soln to a permanent blue color using starch indicator Correct for the quantity of the standard I necessary to produce the same color using the same reagents and volume Calculate the quantity of As_2O_3 present and express the results as percentage of water soluble As_2O_3

TOTAL COPPER OXIDE

30 *Electrolytic Method—Official*

Treat 2 g of the sample in a beaker with 100 cc of H_2O and about 2 g of NaOH and boil thoroly until all the Cu is precipitated as Cu_2O Filter, wash well with hot

H_2O, dissolve the precipitate in hot HNO_3 (1+4), cool, transfer to a 250 cc volumetric flask, and dilute to the mark. Electrolyze an aliquot of 50 or 100 cc as directed under 16. Calculate to percentage of CuO.

31 *Thiosulfate Method*[12]—*Official*

Determine Cu in an aliquot of the HNO_3 soln of Cu_2O, under 30, by titrating with standard thiosulfate soln, as directed under 17, and calculate to percentage of CuO.

LONDON PURPLE

32 MOISTURE—OFFICIAL

Proceed as directed under 2.

TOTAL ARSENIC

Zinc Oxide Sodium Carbonate Method[11]—*Official*

33 REAGENTS

(a) *Zinc oxide sodium carbonate mixture*.—Mix 4 parts of ZnO and 1 part of anhydrous Na_2CO_3.

The other reagents and solns used are described under 3.

34 DETERMINATION

Mix 1 g of the sample thoroly with several times its weight of the ZnO Na_2CO_3 reagent in a shallow porcelain crucible, and cover with a layer of the same reagent. Place the crucible, uncovered, in a muffle and heat gently at first and finally for about 15 min at full heat. (The mass will not sinter.) Cool, transfer to a 500 cc distillation flask and proceed as directed under 5 or 8.

TOTAL ARSENIOUS OXIDE[12]—OFFICIAL

35 REAGENTS

The reagents and solns used are described under 3.

36 DETERMINATION

Dissolve 2 g of the sample in 100 cc of HCl (1+4) at a temp. not exceeding 90°, filter into a 250 cc volumetric flask, wash thoroly, cool, and dilute to volume. Treat 100 cc of this soln with $NaHCO_3$ in excess, transfer to a 500 cc volumetric flask, dilute to the mark, adding a few drops of ether to destroy the bubbles. Mix thoroly and pass thru a dry filter. Titrate 250 cc of the filtrate as directed under 3(b) and calculate the percentage of As_2O_3.

TOTAL ARSENIC OXIDE[13]—OFFICIAL

37 REAGENTS

The reagents and solns used are described under 3.

38 DETERMINATION

Boil on a hot plate or over a low flame 2 g of the sample with 5 cc of HNO_3 and 20 cc of H_2SO_4 in a covered casserole or a Kjeldahl digestion flask. After 10–15 min add fuming HNO_3 or powdered $NaNO_3$ in small quantities at a time until all organic matter is destroyed and the soln is practically colorless. Cool, add about 50 cc

of H_2O (to decompose any nitro sulfuric acid formed), and heat again until all HNO_3 fumes are expelled. Cool, transfer to a 250 cc volumetric flask, dilute to the mark with H_2O, mix thoroly, and filter thru a dry filter.

Transfer 50 cc of this filtrate to a 400 cc Erlenmeyer flask, dilute with H_2O to 100 cc, add 1 g of KI, heat to boiling, and evaporate to about 10 cc (not less). Cool, dilute to 150–200 cc, and remove the excess of I with 0.05 N $Na_2S_2O_3$. If the soln is slightly colored from organic matter or from any materials other than free I add the thiosulfate until it is nearly colorless, then a few drops of the starch indicator, and continue adding the thiosulfate slowly until the blue color just disappears. Continue at once as directed under 3(b), beginning with "neutralize with $NaHCO_3$." Subtract from this reading the number of cc of the standard I soln corresponding to the As_2O_3 obtained under 36, and from the remainder calculate the percentage of As_2O_3 in the sample.

39 WATER SOLUBLE ARSENIOUS OXIDE—OFFICIAL

Proceed as directed under 29, using 2 g of the sample and slightly acidifying the aliquot employed with HCl (1 + 1) before adding the excess of $NaHCO_3$.

WATER SOLUBLE ARSENIC OXIDE—OFFICIAL

40 REAGENTS

The reagents and solns used are described under 3.

41 DETERMINATION

Transfer a 250 cc aliquot of the H_2O extract obtained under 39 to a casserole, add 5 cc of H_2SO_4, evaporate to a small volume, and heat on a hot plate until white fumes of H_2SO_4 appear. Cover the casserole, add 1–2 cc of fuming HNO_3, and again heat until the appearance of white fumes. Cool, add a little H_2O, and in order to expel the last traces of HNO_3, once more evaporate until white fumes appear. Cool, dilute to about 100 cc with H_2O, and add 1 g of KI and sufficient H_2SO_4 to make the total quantity present about 5 cc. Boil until the volume is reduced to about 40 cc. Cool, dilute to about 200 cc, and add the approximately 0.05 N $Na_2S_2O_3$ soln, 9, drop by drop until the I color is exactly removed. Proceed as directed under 3(b), beginning with "neutralize with $NaHCO_3$." Correct for the quantity of the standard I soln necessary to produce the same color using the same reagents and volume. Subtract from the corrected titration reading the number of cc of the standard I soln corresponding to the As_2O_3 obtained under 39, and from the remainder calculate the percentage of As_2O_3 present.

LEAD ARSENATE

42 MOISTURE—OFFICIAL

(a) *Powder.*—Dry 2–5 g to constant weight at 100–110° and report the loss in weight as moisture.

(b) *Paste.*—Proceed as directed under (a), using 10 g. Grind the dry sample to a fine powder, mix well, transfer a small portion to a sample bottle, and again dry for 1–2 hours at 100–110°. Use this anhydrous material for the determination of total H_2O as directed under 5 or 8.

TOTAL ARSENIC

43 METHOD OF ANALYSIS

Proceed as directed under 5 or 8.

METHODS OF ANALYSIS

Method II[14]*—Official*

(Not applicable in the presence of antimony)

44

REAGENTS

The reagents and solns used are described under *3*

45

DETERMINATION

Dissolve 1 g of the powdered sample in HNO_3 (1+4) in a porcelain casserole or evaporating dish, add 5 cc of H_2SO_4 and heat on a hot plate to copious evolution of white fumes Cool, add a little H O and again evaporate until the appearance of white fumes to assure removal of the last trace of HNO_3 Wash into a 200 cc volumetric flask with H_2O, cool dilute to volume, and filter thru a dry filter Transfer 100 cc of the filtrate to an Erlenmeyer flask and proceed as directed under *13*, beginning with "add 1 gram of KI ' From the number of cc of standard I soln used calculate the percentage of total As in the sample in terms of As_2O_3

46

TOTAL ARSENIOUS OXIDE[14]—OFFICIAL

REAGENTS

The reagents and solns used are described under *3*

47

DETERMINATION

Weigh 2 g of the powdered sample and transfer to a 200 cc volumetric flask, add 100 cc of H_2SO_4 (1+6), and boil for 30 min Cool dilute to volume shake thoroly, and filter thru a dry filter Nearly neutralize 100 cc of the filtrate with NaOH soln, *3(d)*, using a few drops of phenolphthalein indicator If the neutral point is passed, make acid again with the dilute H_2SO_4 Continue as directed under *3(b)*, beginning "neutralize with $NaHCO_3$ ' From the number of cc of standard I soln used calculate the percentage of As_2O_3

48

TOTAL ARSENIC OXIDE[14]—TENTATIVE

REAGENTS

(a) *Starch indicator* —Prepare as directed under *3(e)*

(b) *Ammonium chloride soln* —Dissolve 250 g of NH_4Cl in H_2O and dilute to 1 liter

(c) *Potassium iodide soln* —Dissolve 20 g of KI in H_2O and dilute to 100 cc

(d) *Standard iodine soln* —Prepare as directed under *3(b)* but calculate in terms of As O_3

(e) *Standard thiosulfate soln* —Prepare an approximately 0 05 *N* soln as follows Dissolve 13 g of $Na_2S_2O_3$ $5H_2O$ in recently boiled and cooled H_2O filter, and dilute to 1 liter with recently boiled and cooled H_2O Standardize as follows Dissolve about 0 7 g of $PbHAsO_4$ in 50 cc of HCl in an Erlenmeyer flask If necessary to effect soln heat on a steam bath keeping the flask covered with a watch glass to prevent evaporation of the acid Cool to 20–25 add 10 cc of the KI soln (c) and 50 cc (or more if necessary to produce a clear soln) of the NH_4Cl soln, *(b)*, and immediately titrate the liberated I with the standard thiosulfate When the color becomes a faint yellow, dilute with about 150 cc of H_2O and continue the titration carefully, dropwise, until colorless using the starch indicator near the end point From the weight of $PbHAsO_4$ and the number of cc of $Na_2S_2O_3$ soln used calculate the value of the latter in terms of As_2O_3 (As O_4 in $PbHAsO_4$ = 33 11 per cent)

Pure $PbHAsO_4$ may be prepared by pouring a soln of $Pb(NO_3)_2$ into a soln of KH_2AsO_4 which should be in excess Collect the precipitate by filtration, dissolve it in the smallest possible quantity of boiling HNO_3 (1+4), and pour the soln into a large quantity of H_2O Collect the precipitate by filtration and dry at 110°

49 DETERMINATION

Weigh 0 5 g of the powdered sample and transfer to an Erlenmeyer flask Add 25–30 cc of HCl and evaporate to dryness on a steam bath Then add 50 cc of HCl and proceed as directed under 48(e), beginning with "If necessary to effect soln, heat on a steam bath" From the number of cc of standard thiosulfate soln used calculate the percentage of As_2O_5

50 WATER SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 13, and calculate results as As_2O_5

TOTAL LEAD OXIDE

51 *Method I*[12]*—Official*

Heat, on a hot plate, 0 5 g of the powdered sample with about 25 cc of HNO_3 (1+4) in a 600 cc beaker Remove any insoluble residue by filtration Dilute to at least 400 cc heat nearly to boiling, and add NH_4OH to slight precipitation, then HNO_3 (1+9) to redissolve the precipitate adding 1–2 cc in excess Pipet into this soln, kept almost boiling, 50 cc of a hot 10% K_2CrO_4 soln stirring constantly Decant while hot thru a weighed Gooch crucible, previously heated to 140–150° and wash several times by decantation and then on the filter with boiling H_2O until the wash ings are colorless Dry the $PbCrO_4$ at 140–150° to constant weight From the weight of the $PbCrO_4$ calculate the percentage of PbO in the sample, using the factor 0 6906

The $PbCrO_4$ precipitate may contain a small quantity of $PbHAsO_4$ which will cause slightly high results, but this error rarely amounts to more than 0 1–0 2%

52 *Method II*[13]*—Official*

(Not applicable in the presence of calcium)

Heat, on a hot plate 0 5 g of the powdered sample with about 25 cc of HNO_3 (1+4) in a porcelain evaporating dish or casserole Remove any insoluble residue by filtration Add 3 cc of H_2SO_4 and evaporate on a hot plate until the appearance of white fumes Cool, add a few cc of H_2O (to decompose any nitro sulfuric acid formed) and again heat to fuming Proceed as directed under 15, beginning with "Cool add 50 cc of H_2O and 100 cc of 95% alcohol

CALCIUM ARSENATE

53 MOISTURE—OFFICIAL

Proceed as directed under 2

54 TOTAL ARSENIC

Proceed as directed under 5 or 8

TOTAL ARSENIOUS OXIDE[10]—OFFICIAL

55 REAGENTS

The reagents and solns used are described under 3

56 DETERMINATION

(a) (Not applicable in the presence of nitrates)

this point by adding a little $NaHSO_3$ and heating on a steam bath until the odor of SO_2 has largely disappeared Cool, dilute to about 100 cc, and proceed as directed under **19**, beginning with "add 35–40 cc of the Hg thiocyanate reagent with vigorous stirring"

COPPER CARBONATE

COPPER OXIDE

74 *Electrolytic Method—Official*

Weigh 0 5 g of the sample, transfer to a 150 cc Pt dish or 150 cc beaker, and dissolve in 25 cc of HNO_3 (1+4) Dilute to about 100 cc and determine the Cu by electrolysis, as directed under **16**, beginning with "electrolyze, using a rotating anode and a current of about 3 amperes"

75 *Thiosulfate Method—Official*

Dissolve 0 25–0 5 g of the sample in 25 cc of HNO_3 (1+4), dilute to about 50 cc, and proceed as directed under **17**, beginning with "add NH_4OH in excess"

BORDEAUX MIXTURE

MOISTURE—OFFICIAL

76 (a) *Powder*—Dry 2 g to constant weight at 105–110° and report the loss as moisture

(b) *Paste*—Heat about 100 g in an oven at 90–100 until dry enough to powder readily and note the loss in weight *Powder this partially dried sample and determine* the remaining moisture in 2 g, as directed under (a) Determine CO_2 as directed under **78**, both in the original paste and in this partially dried sample Calculate the total moisture by the following formula

$$M = a + \frac{(100-a)(b+c)}{100} - d, \text{ in which}$$

M = percentage of total moisture in original paste,
a = percentage of loss in weight of original paste during first drying,
b = percentage of loss in weight of partially dried paste during second drying,
c = percentage of CO_2 remaining in partially dried paste after first drying, and
d = percentage of total CO_2 in original paste

CARBON DIOXIDE[20]—OFFICIAL

77 APPARATUS

Use a 200 cc Erlenmeyer flask closed with a 2-holed stopper, one hole is fitted with a dropping funnel, the stem of which extends almost to the bottom of the flask, and the outlet of a condenser, which is inclined upward at an angle of 30° from the horizontal, passes downward thru the other hole The upper end of the condenser is connected with a CaCl tube which in turn is connected with a double U tube filled in the middle with pumice fragments, previously saturated with $CuSO_4$ soln (20% $CuSO_4 \cdot 5H_2O$) and subsequently dehydrated, and with CaCl at either end Then follow two weighed U tubes for absorbing the CO_2 the first filled with porous soda lime, and the second, $\frac{1}{3}$ with soda lime and $\frac{2}{3}$ with $CaCl_2$, the latter reagent being placed at the exit end of the train A Geissler bulb, partly filled with H_2SO_4 is attached to the last U-tube to show the rate of gas flow An aspirator is connected with the Geissler bulb to draw air thru the apparatus An absorption tower filled with

soda lime is connected with the mouth of the dropping funnel to remove CO_2 from the air entering the apparatus

78 DETERMINATION

Weigh 2 g of the powder or 10 g of the paste into the Erlenmeyer flask, add about 20 cc of H_2O, attach the flask to the apparatus, omitting the 2 weighed U-tubes, and draw CO_2 free air thru the apparatus until the original air is displaced Then attach the weighed U tubes in position, as described under 77, close the stopcock of the dropping funnel, pour into it 50 cc of HCl (1+4), reconnect with the soda lime tower, and allow the acid to flow into the Erlenmeyer flask, slowly if there is much CO_2, rapidly if there is little When effervescence diminishes place a low Bunsen flame under the flask and start a flow of H O thru the condenser, allowing a slow current of air to flow thru the apparatus at the same time Maintain a steady but quiet ebullition and a slow air current thru the apparatus Boil for a few min after the H_2O has begun to condense in the condenser, then remove the flame and continue the aspiration of air at the rate of about 2 bubbles per second until the apparatus is cool Disconnect the weighed absorption tubes cool in the balance case and weigh The increase in weight is CO_2

COPPER

79 *Electrolytic Method—Official*

Dissolve 2 g of the powdered sample in 25 cc of HNO_3 (1+4), dilute to 100 cc, and electrolyze, using a rotating spiral anode and a current of about 3 amperes as directed under 16, beginning with "Wash into a weighed 150 cc Pt dish"

80 *Thiosulfate Method—Official*

Dissolve 2 g of the powdered sample in about 25 cc of HNO_3 (1+4) dilute to 50 cc, add NH_4OH in excess and heat, then, without removing the precipitate which is formed, boil off the excess of NH_3 add 3–4 cc of acetic acid, cool, add 10 cc of 30% KI soln, and titrate as directed under 17, beginning with "titrate with standard thiosulfate soln"

BORDEAUX MIXTURE WITH PARIS GREEN

81 MOISTURE—OFFICIAL

Proceed as directed under 76

82 CARBON DIOXIDE—OFFICIAL

Proceed as directed under 78

83 TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of the sample and calculating the results as As_2O_3

84 TOTAL ARSENIOUS OXIDE—OFFICIAL

Proceed as directed under 23, using 0 5–1 0 g of the sample

85 WATER SOLUBLE ARSENIOUS OXIDE—OFFICIAL

Proceed as directed under 29, using 2 g of the sample and slightly acidifying the aliquot employed with HCl (1+4) before adding the excess of $NaHCO_3$

COPPER

Electrolytic Method I—Official

86 Proceed as directed under 16

Electrolytic Method II—Official

87 Dissolve 2 g of the powdered sample in a 150 cc beaker with 5 cc of HNO_3, add 25 cc of a 3% soln of H_2O_2, and warm on a steam bath for 5–10 min Add 25 cc more of H_2O_2 soln, dilute to 100 cc, and electrolyze, using a weighed gauze cathode, a rotating paddle anode, and a current of 2–3 amperes At the end of about 20 min add 15–20 cc more of the H_2O_2 soln After all Cu is deposited, which should not require more than 45 min and while the current is still flowing, wash the deposit with H_2O by siphoning Then interrupt the current, rinse with alcohol, dry for a few min in an oven, weigh, and calculate the percentage of Cu (Do not pass the current for more than 5–10 min after all Cu is deposited without adding more of the H_2O_2 soln)

Thiosulfate Method—Official

88 Proceed as directed under 17

BORDEAUX MIXTURE WITH LEAD ARSENATE

MOISTURE—OFFICIAL

89 Proceed as directed under 76

CARBON DIOXIDE—OFFICIAL

90 Proceed as directed under 78

TOTAL ARSENIC—OFFICIAL

91 Proceed as directed under 5 or 8, using 2 g of the sample and calculating the results as As_2O

WATER SOLUBLE ARSENIC—OFFICIAL

92 Proceed as directed under 13 and calculate the results as As_2O_5

COPPER

Electrolytic Method—Official

93 Proceed as directed under 16

Thiosulfate Method—Official

94 Proceed as directed under 17

LEAD OXIDE—OFFICIAL

95 Proceed as directed under 15

BORDEAUX MIXTURE WITH CALCIUM ARSENATE

MOISTURE—OFFICIAL

96 Proceed as directed under 76

CARBON DIOXIDE—OFFICIAL

97 Proceed as directed under 78

95 TOTAL ARSENIC—OFFICIAL

Proceed as directed under 5 or 8, using 2 g of the sample and calculating the results as As_2O_3.

99 WATER SOLUBLE ARSENIC—OFFICIAL

Proceed as directed under 13 and calculate the results as As_2O_3.

COPPER

100 *Electrolytic Method I—Official*

Proceed as directed under 16.

101 *Electrolytic Method II—Official*

Proceed as directed under 87.

102 *Thiosulfate Method—Official*

Proceed as directed under 17.

SODIUM AND POTASSIUM CYANIDES

CYANOGEN—OFFICIAL

103 REAGENTS

(a) *Silver nitrate solution*—0.1 N. Standardize against pure NaCl by titration using chromate as indicator or gravimetrically, weighing the chloride.

(b) *Indicator*—

(c) *Sodium hydroxide*—Dissolve 100 g of NaOH in H_2O and dilute to 1 liter.

(d) *Potassium iodide*—Crystals or a saturated soln.

104 DETERMINATION

Break the sample to a small lump in a mortar (do not grind). Weigh quickly [illegible] g, transfer to the bottle of a Wash bottle to a 250-cc volumetric flask containing about 200 cc of H_2O. Add a little H_2O, to precipitate any salts that may be present. Shake to dissolve and wash H_2O into the flask and fill the mark. Transfer a [illegible] cc aliquot to a beaker, add 100 cc of H_2O, 5 cc of the NaOH soln. and 10 cc of the KI soln., titrate a few cc at a time with the 0.1 N $AgNO_3$ (decreasing the intervals as it is necessary to have the beaker contents shake after the addition of the $AgNO_3$) to the first appearance of a faint opalescence. The reaction is represented by the equation: $2NaCN + AgNO_3 = NaAg(CN)_2 + NaNO_3$. Hence 1 cc of 0.1 N $AgNO_3$ solution = [illegible] g of CN.

CHLORINE

Method I—Official

105 PREPARATION

[illegible]

106 DETERMINATION

Transfer a 50 cc aliquot of the soln prepared as directed under 104 to a beaker dilute with an equal volume of H_2O, add 1–2 cc of the HCHO soln, stir well, and let stand for 15 min Acidify with HNO_3 (1+1) (5 cc is usually enough), add a measured volume of the 0 1 *N* $AgNO_3$ soln sufficient to give an excess, stir well, filter, wash, and titrate the excess of Ag in the combined filtrate and washings with the 0 1 *N* thiocyanate soln, using ferric indicator From the number of cc of 0 1 *N* $AgNO_3$ soln less the number of cc of 0 1 *N* thiocyanate soln used, calculate the percentage of Cl in the sample

Method II[2]*—Official*

107 REAGENTS

The reagents and solns used are described under 105

108 DETERMINATION

Transfer a 50 cc aliquot of the soln prepared as directed under 104 to a distilling flask, dilute to 100–150 cc, acidify with a slight excess of acetic acid, and distil, passing the vapors thru a condenser, the delivery end of which dips into a soln of NaOH, to absorb the HCN After all the HCN has been driven off, which should be the case when 50 cc of distillate has passed over, wash the liquid remaining in the distilling flask into a beaker, add 5 cc of HNO_3 (1+1) and then a measured volume of the 0 1 *N* $AgNO_3$ soln sufficient to give an excess, stir well, filter, wash, and titrate the excess of Ag in the combined filtrate and washings with the 0 1 *N* thiocyanate soln using ferric indicator From the number of cc of 0 1 *N* $AgNO_3$ soln, less the number of cc of 0 1 *N* thiocyanate soln used, calculate the percentage of Cl in the sample

CALCIUM CYANIDE

CYANOGEN[23]—OFFICIAL

109 REAGENTS

(a) *Silver nitrate soln* —0 1 *N* Prepare as directed under 103(a)

(b) *Soda lead* —Dissolve 20 g of Pb acetate in H_2O dilute to 1 liter, and add 200 g of chloride free Na_2CO_3

(c) *Sodium hydroxide soln* —Prepare as directed under 103(c)

(d) *Potassium iodide* —Crystals, or a saturated soln

110 DETERMINATION

Place about 200 cc of H_2O in a 500 cc volumetric flask and carefully dry the neck of the flask Weigh about 5 g of the sample in a weighing bottle and transfer to the flask with the least possible exposure to the air Wash the mixture down into the flask and mix by whirling until soln is complete and the small quantity of CaC_2 has been decomposed Then add 25 cc of the soda lead reagent, or a quantity sufficient to remove sulfides close the flask with a rubber stopper, and shake thoroly preferably for half an hour Dilute to the mark, mix and filter thru a dry filter Transfer a 50 cc aliquot to a 400 cc beaker and proceed as directed under 104, beginning with "Add 200 cc of H_2O " 1 cc of 0 1 *N* $AgNO_3$ soln = 0 005202 g of CN If the percentage of $Ca(CN)_2$ is desired, multiply the percentage of CN by the factor 1 7703

INSECTICIDES AND FUNGICIDES

CHLOPINE*

Method I—Official

REAGENTS

111 The reagents and apparatus used are described under 105.

DETERMINATION

112 Transfer a 50 cc aliquot of the soln prepared as directed under 110 to a beaker, and proceed as directed under 106.

Method III—Official

REAGENTS

113 The reagents and apparatus used are described under 105.

DETERMINATION

114 Transfer a 50 cc aliquot of the soln prepared as directed under 110 to a distilling flask and proceed as directed under 108.

SOAP

MOISTURE—OFFICIAL

Xylene Distillation Method

115 REAGENTS

(a) Xylene.—The technical grade is satis[illegible] [illegible] the form of [illegible]

[illegible]

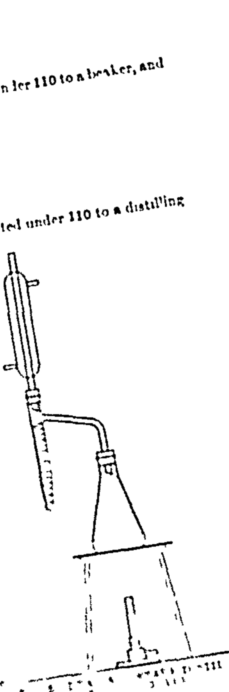
DETERMINATION

116 [illegible]

[illegible] OFFICIAL

117 [illegible]

[illegible] XII 16



MINERAL OILS

[illegible]

XIII 9

118 [illegible]

DETERMINATION

With a pipet measure 5 cc of the oil into a Babcock cream bottle about 15 cm (6 inches) long, either the 9 g 50% or the 18 g 30% type With heavy oils, to reduce the viscosity, warm the pipet after a preliminary draining by drawing it several times thru the flame of a Bunsen burner and then drain thoroly If greater accuracy is desired weigh the measured charge and calculate its exact volume from the weight and sp gr of the oil Add slowly 20 cc of the 38 N H_2SO_4, gently shaking or rotating the bottle and taking care that the temp does not rise above 60° Cool in ice H_2O if necessary When the mixture no longer develops heat on shaking agitate thoroly, place the bottle in a water bath, and heat at 60–65° for 10 min, keeping the contents of the bottle thoroly mixed by shaking vigorously for a period of 20 seconds at 2 min intervals Remove the bottle from the bath and fill with H_2SO_4 until the oil rises into the graduated neck Centrifugalize for 5 min (or longer if necessary to obtain a constant volume of oil) at 1200–1500 r p m Read the volume of unsulfonated residue from the graduations on the neck of the bottle and, to convert to cc, multiply the reading from the 9 g 50% bottle by 0 1 and that from the 18 g 30% bottle by 0 2 From the result thus obtained calculate the percentage by volume of the unsulfonated oil

120 MINERAL OIL—SOAP EMULSIONS

WATER[a]

Xylene Distillation Method—Official

Weigh about 25 g of the sample into a 300–500 cc flask, add 50 cc of xylene and if necessary to prevent foaming, a small piece of rosin, and proceed as directed in 116, beginning with 'Distil into a Dean and Stark distilling tube receiver'

121

TOTAL OIL[a]—OFFICIAL

Weigh about 10 g of the sample into a Babcock cream bottle Dilute with about 10 cc of hot H_2O and add 5–10 cc of H_2SO_4(1+1) Set the bottle in a hot water bath for about 5 min to hasten the separation of the oil, add sufficient saturated NaCl soln to bring the oil layer within the graduations on the neck of the bottle whirl at a rate of 1200 r p m for 5 min and allow to cool Read the volume of the oil layer determine its density and from these values calculate its weight and percentage From this percentage value deduct the percentage of fatty acids (and phenols if present) determined separately to obtain the percentage of oil in the sample

122

SOAPS—OFFICIAL

(In this method error will result if the apparent molar weight of the fatty acids varies appreciably from that of oleic acid)

Weigh 20 g of the sample into a separatory funnel, add 60 cc of petroleum ether and extract the mixture once with 20 cc and 4 times with 10 cc of 50% alcohol Break the emulsion if necessary with 1 or 2 cc of a strong soln of NaOH allowing the soln to run down the side of the separatory funnel which is then gently twirled and allowed to stand for a few min Draw off the alcoholic layers and wash them successively thru petroleum ether contained in 2 other separatory funnels Combine the alcoholic extracts in a beaker and evaporate on a steam bath to remove the alcohol Dissolve the residue in about 100 cc of H_2O made alkaline with NaOH Transfer to a separatory funnel acidify with HCl or H_2SO_4 extract 3 times with ether and wash the ether extracts twice with H_2O Combine the ether extracts evaporate in a weighed beaker on a steam bath and weigh as fatty acids From the weight of fatty acids calculate the percentage of soap in the sample as Na or K-oleate

123 UNSULFONATED RESIDUE—OFFICIAL

Determine on 5 cc of the recovered oil, as described under Mineral Oils 119

124 ASH—OFFICIAL

Evaporate 10 g of the sample, or more if necessary, in a Pt dish, ignite, and leach the charred mass with H_2O. Ignite the residue, add the leachings, evaporate to dryness, ignite, and weigh. From this weight calculate the percentage of ash. Test the ash for Cu, Ca, CaF_2, etc.

SODA LYE

CARBONATE AND HYDROXIDE—OFFICIAL

125 REAGENTS

(a) *Hydrochloric acid*.—0.5 *N*. Prepare and standardize as directed under II, 17(a).

(b) *Methyl orange indicator*.—Prepare as directed under 3(f).

(c) *Phenolphthalein indicator*.—Dissolve 1 g of phenolphthalein in 100 cc of neutralized alcohol 95% by volume.

(d) *Barium chloride soln*.—Dissolve 100 g of $BaCl_2 \cdot 2H_2O$ and dilute to 1 liter.

126 DETERMINATION

Weigh about 10 g of the sample from a weighing bottle, dissolve in CO_2-free H_2O, and dilute to a definite volume. Titrate an aliquot of this soln with the 0.5 *N* HCl, using the methyl orange indicator, and note the total alkalinity thus found. Transfer an equal aliquot to a volumetric flask and add enough of the $BaCl_2$ soln to precipitate all the carbonate, avoiding any unnecessary excess. Dilute to the mark with CO_2-free H_2O, stopper, shake, and set aside. When the liquid becomes clear, pipet off a portion and titrate with the 0.5 *N* HCl, using the phenolphthalein indicator. The number of cc of 0.5 *N* acid required for this titration, multiplied by 2, gives the number of cc of 0.5 *N* acid equivalent to the NaOH present in the original aliquot. The difference between this figure and the number of cc of 0.5 *N* HCl required for the total alkalinity represents the number of cc of 0.5 *N* acid equivalent to the Na_2CO_3 present in the aliquot. Calculate the percentages of Na_2CO_3 and NaOH present in the sample.

TOBACCO AND TOBACCO EXTRACT

NICOTINE

Silicotungstic Acid Method—Official

127 REAGENTS

(a) *Silicotungstic acid soln*.—Dissolve 120 g of silicotungstic acid ($4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 22H_2O$) in H_2O and dilute to 1 liter. (There are several silicotungstic acids. The acids $4H_2O \cdot SiO_2 \cdot 10WO_3$, $3H_2O$ and $4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 20H_2O$ do not give crystalline precipitates with nicotine and should not be used.)

(b) *Sodium hydroxide soln*.—Dissolve 400 g of NaOH in H_2O and dilute to 1 liter.

(c) *Hydrochloric acid*.—(1 + 1).

128 DETERMINATION

Weigh such a quantity of the preparations as will contain preferably between 0.1 and 1.0 g of nicotine (if the sample contains very little nicotine, i.e. about 0.1%, do

not increase the quantity to the point where it interferes with the distillation), wash with H_2O into a 500 cc round-bottomed distillation flask, and add a little paraffin to prevent frothing, a few small pieces of pumice, and a slight excess of the NaOH soln, using the phenolphthalein indicator Distil rapidly in a current of steam thru a well cooled condenser connected by means of an adapter with a suitable flask containing 10 cc of the dilute HCl When distillation is well under way, heat the distillation flask to reduce the volume of the liquid as far as practicable without bumping or undue separation of insoluble matter Distil until a few cc of the distillate shows no cloud or opalescence when treated with a drop of the silicotungstic acid and a drop of the dilute HCl Confirm the alkalinity of the residue in the distillation flask with the phenolphthalein indicator Make the distillate, which may amount to 1000–1500 cc, to a convenient volume (the soln may be concentrated on the steam bath *without loss of nicotine*), *mix well, and pass thru a large dry filter if not clear* Test a portion with methyl orange to confirm its acidity Pipet an aliquot, containing about 0 1 g of nicotine, into a beaker (if the samples contain very small quantities of nicotine, an aliquot containing as little as 0 01 g of nicotine may be used), add to each 100 cc of liquid 3 cc of the dilute HCl, or more if the necessity is indicated by the test with methyl orange, and 1 cc of the silicotungstic acid for each 0 01 g of nicotine supposed to be present Stir thoroly and let stand overnight Before filtering, stir the precipitate to see that it settles quickly and is in crystalline form, filter on an ashless filter, and wash with cold HCl (1+1000) Transfer the paper and precipitate to a weighed Pt crucible, dry carefully, and ignite until all C is destroyed Finally heat over a Teclu or Meker burner for not more than 10 min The weight of the residue, multiplied by 0 1140, gives the weight of nicotine present in the aliquot

FORMALDEHYDE SOLUTIONS

FORMALDEHYDE

Hydrogen Peroxide Method[33]*—Official*

129 REAGENTS

(a) *Sulfuric acid*—1 *N* Prepare as directed under II, 17(b)

(b) *Sodium hydroxide*—1 *N* Standardize against (a), using *the litmus or brom* thymol blue indicator 1 cc = 30 02 mg of HCHO

(c) *Hydrogen peroxide*—The commercial reagent containing about 3% of H_2O_2 If acid, neutralize with the 1 *N* NaOH (b), using litmus or brom thymol blue indicator

(d) *Litmus indicator*—A soln of purified litmus of sufficient strength that 3 drops will impart a distinct blue color to 50 cc of H_2O

(e) *Brom thymol blue indicator*—Dissolve 1 g of brom thymol blue in 500 cc of alcohol, 50% by volume

130 DETERMINATION

Measure 50 cc of the 1 *N* NaOH into a 500 cc Erlenmeyer flask and add 50 cc of the H_2O_2 Then add a weighed quantity, about 3 g of the HCHO soln under examination, allowing the point of the weighing pipet to reach nearly to the liquid in the flask Place a funnel in the neck of the flask and heat on a steam bath for 5 min, shaking occasionally Remove from the steam bath, wash the funnel with H_2O, cool the flask to about room temp, and titrate the excess NaOH with 1 *N* acid, using the brom thymol blue or litmus indicator (It is necessary to cool the flask before titration with the acid in order to obtain a sharp end point with the litmus) *From the amount of* 1 *N* NaOH *consumed* and the weight of the sample calculate the percentage of HCHO, according to the following equation

$$NaOH+HCHO+H_2O_2=NaCOOH+2H_2O$$

Cyanide Method[a]*—Official*

(Applicable only to dilute solutions)

REAGENTS

131 (a) *Silver nitrate*—0 1 *N* Prepare as directed under XII, 34(a)
(b) *Ammonium thiocyanate*—0 1 *N* Prepare as directed under XII, 34(b)
(c) *Potassium cyanide soln*—Dissolve 3 1 g of KCN in 500 cc of H_2O

DETERMINATION

132 Treat 15 cc of the 0 1 *N* $AgNO_3$ soln with 6 drops of HNO_3 (1+1) in a 50 cc volumetric flask add 10 cc of the KCN soln, dilute to the mark, shake well, filter thru a dry filter, and titrate 25 cc of the filtrate with the 0 1 *N* NH_4SCN, as directed under XII, 35 Acidify another 15 cc portion of the 0 1 *N* $AgNO_3$ with 6 drops of the dilute HNO_3 and treat with 10 cc of the KCN soln to which has been added a measured quantity (the weight must be calculated from the sp gr) of the HCHO soln containing not over 25 mg of HCHO Dilute to 50 cc, filter and titrate a 25 cc aliquot with the 0 1 *N* NH_4SCN for the excess of Ag as before The difference between the number of cc of NH_4SCN used in these 2 titrations multiplied by 2 gives the number of cc of 0 1 *N* NH_4SCN corresponding to the KCN used by the HCHO Calculate the percentage of HCHO present 1 cc of 0 1 *N* NH_4SCN = exactly 3 mg of HCHO

LIME-SULFUR SOLUTIONS

TOTAL SULFUR[a]—OFFICIAL

PREPARATION OF SAMPLE

133 Weigh about 10 g of the soln, transfer to a 250 cc volumetric flask, and immediately dilute to the mark with recently boiled and cooled H_2O Mix thoroly and transfer to a number of small bottles filling them completely and avoiding contact of the soln with air as much as possible Stopper the bottles seal with paraffin, and preserve in a dark cool place

DETERMINATION

134 (Sulfur free reagents should be used for all work)

Dissolve 2–3 g of Na_2O_2 in 50 cc of cold H_2O in a 200 cc beaker Transfer a 10 cc aliquot of the soln prepared for analysis as directed under 133, to this aqueous soln of Na_2O_2, keeping the tip of the pipet constantly just under the surface of the liquid until nece sary to raise it for drainage at the end Use a clean dry pipet for measuring each portion Cover the beaker with a watch glass and heat on a steam bath, with occasional stirring until all the S is oxidized to sulfate (indicated by the disappearance of the yellow color) Wash off the watch glass and the sides of the beaker, acidify with HCl (1+1), evaporate to complete dryness treat with H_2O acidified with HCl boil and filter to remove SiO_2 Dilute the filtrate to 300 cc add 50 cc of HCl, heat to boiling and add 10 cc $BaCl_2$ soln slowly and with constant stirring (The $BaCl_2$ should be added at such a rate that about 1 min is required for running in the nece sary quantity 11 cc for 1 g of $BaSO_4$ The rate is best regulated by attaching a suitable capillary tip to a buret containing the $BaCl_2$ soln) Evaporate to dryness on a steam bath take up with hot H_2O filter thru a quantitative filter wash until free from chlorides ignite carefully and heat to constant weight over a

Bunsen burner Calculate the percentage of S from the weight of $BaSO_4$, using the factor 0 1374

MONOSULFIDE EQUIVALENT[6]—TENTATIVE

135 REAGENT

Iodine soln —0 1 *N* Prepare as directed under 3(b), using 12 7 g of I and 25 g of KI

136 DETERMINATION

Dilute 10 cc of the soln, prepared as directed under 133, to about 30 cc with recently boiled and cooled H_2O and titrate with the 0 1 *N* I soln until the yellow color just disappears (There should be no difficulty in determining this end point, if there is, a small crystal of Na nitroprusside may be used It must not be added until the end point is practically reached, since the blue color, if well developed, cannot be destroyed except by an excess of I) From the number of cc of 0 1 *N* I used calculate the percentage of monosulfide equivalent 1 cc of 0 1 *N* I = 0 0016 g of S as monosulfide equivalent

THIOSULFATE SULFUR

Zinc Chloride Method[35]*—Official*

137 REAGENT

Ammoniacal zinc chloride soln —Dissolve 50 g of pure $ZnCl_2$ in about 500 cc of H_2O, add 125 cc of NH_4OH and 50 g of NH_4Cl and dilute to 1 liter

138 DETERMINATION

To 50 cc of H_2O in a 200 cc volumetric flask add, in the manner indicated under 134, 50 cc of the soln prepared as directed under 133 Add a slight excess of the ammoniacal $ZnCl_2$ soln and dilute to the mark Shake thoroly and filter thru a dry filter To 100 cc of the filtrate add a few drops of methyl orange or methyl [illegible] cator and exactly neutralize with 0 1 *N* HCl Titrate the neutral soln with approximately 0 05 *N* I soln, 3(b), using a few drops of the starch indicator or bromthe number of cc of I soln used calculate the percentage of thiosulfate S % of H_2O_3 the value of the I soln is given in terms of As O_3 it is necessary to multipl[illegible] by 1 296 to obtain the equivalent of thiosulfate S

139 *Iodine Titration Method*[35]*—Tentative* [illegible] that 3 drops

Continue the titration of the soln used in the determination of th[illegible] equivalent, 136 with the 0 1 *N* I soln, letting the I act as its own in[illegible] in 500 cc of small drop produces a slight permanent coloration From the numbe[illegible] I used calculate the percentage of thiosulfate S 1 cc of 0 1 *N* I = 0[illegible] thiosulfate

add 50 cc of [illegible]oln under ex[illegible] to the liquid [illegible]n bath for 5

SULFIDE SULFUR

140 *Zinc Chloride Method*[35]*—Official*

To 10–15 cc of H_2O in a small beaker add, in the manner indicated [illegible] funnel with 10 cc aliquot of the soln prepared as directed under 133 Calculate [illegible] with 1 *N* of ammoniacal $ZnCl_2$ soln 137, necessary to precipitate all the S in the a[illegible] cool the add a slight excess Stir thoroly filter, wash the precipitate twice with [illegible] with the and transfer the filter paper and precipitate to the beaker in which the pre[illegible] sample was made Cover with H_2O, disintegrate the paper with a glass rod, and ac[illegible]

3 g of Na_2O_2, keeping the beaker well covered with a watch glass Warm on a steam bath with frequent shaking until all the S is oxidized to sulfate adding more Na_2O_2 if necessary Make slightly acid with HCl (1+4), filter to remove shreds of the filter paper, wash thoroly with hot H_2O, and determine the S in the filtrate as directed under 134

Iodine Titration Method[28]*—Tentative*

141 Allow the soln from 139 to stand several hours with occasional stirring, or acidify with a few drops of HCl (1+4), warm gently with stirring filter, and wash thoroly with warm H_2O Place the filter paper with the S in a small vessel and dissolve the S in about 15 cc of NaOH soln, 3(d), by heating gently on a steam or water bath for 1–1 5 hours (do not boil) Keep the flask covered and shake gently a few times during the digestion to remove the S from the sides Oxidize by adding 2–3 g of Na_2O_2 dissolved in 50 cc of cold H_2O and complete the determination as directed under 134, beginning with ' Cover the beaker with a watch glass

Indirect Method—Tentative

142 The difference between the total S and the sum of the thiosulfate S and sulfate S is the sulfide S

SULFATE SULFUR

Zinc Chloride Method—Official

143 Slightly acidify the soln from the determination of thiosulfate S 138, with HCl (1+4), heat to boiling add slowly and with constant stirring a slight excess of a 10% $BaCl_2$ soln, boil for 30 min allow to stand overnight filter, calculate the S from the weight of $BaSO_4$ and report as % of sulfate S

Iodine Titration Method[28]*—Tentative*

144 To the filtrate from the determination of thiosulfate S 139, add several drops of ~~Na~~—Cl precipitate in the cold with 5 cc of a 10% $BaCl_2$ soln, allow to stand overnight, transfer t alculate the S from the weight of $BaSO_4$ and report as percentage of sulfate of the soln preserve in

TOTAL LIME[28]—OFFICIAL

134 c of the soln prepared as directed under 133, add 10 cc of HCl, evaporate 3 on a steam bath treat with H O and a few cc of HCl (1+4) warm until Cl_2 is dissolved and filter to remove S and any SiO_2 that may be present Dissolve e filtrate to a volume of 200–250 cc heat to boiling add a few cc of NH_4OH aliquot of t and then an excess of a saturated soln of $(NH_4)_2C_2O_4$ Continue the boiling of Na_2O_2 precipitated CaC_2O_4 assumes a well defined granular form, allow to stand until nece ur, filter, and wash a few times with hot H_2O Ignite in a Pt crucible over a ing each p ip to constant weight and calculate to percentage of CaO with occasi pearance of

FLUORINE

By Volatilization[28]*—Tentative*

REAGENTS

s acidify with with HCl bo HCl, heat to

The $BaCl_2$ *uric acid*—98–98 5% Prepare by one of the following methods in the nec dd sufficient fuming H_2SO_4 to ordinary concentrated H_2SO_4 to give a soln attaching ing about 99% as determined by titration Heat in an open casserole for 1 to dryne ter the appearance of dense fumes Adjust the acid to contain 98–98 5% by wash ur

ignition at a dull red heat under a hood having a good draught (The crucible will melt in the full heat of a Bunsen burner)

Precipitation Method[38]—*Official, First Action*

153 REAGENTS

(a) *Hydrogen peroxide*—A 30% soln, commonly designated as "perhydrol" or "superoxol"

(b) *Potassium permanganate soln*—Approximately 0 1 *N*

154 APPARATUS

Digestion flask.—A 200 cc Erlenmeyer flask, fitted with an air condenser by means of a ground glass joint

155 DETERMINATION

Place 0 5–2 0 g of the sample, depending on the quantity of Hg present, in the digestion flask Add 10 cc of H_2SO_4, connect the flask to the condenser, and rotate in order to bring all the sample into contact with the acid Then add dropwise thru the condenser tube 3–5 cc of the 30% H_2O_2 soln, and mix by rotation of the flask After the active reaction has subsided, heat over a low flame for 15–20 min, add 5 cc more of the H_2O_2 and continue the heating until all organic matter is destroyed (indicated by a clear soln), adding more H_2O_2 if necessary Remove the flask from the heat allow to cool, wash down the condenser, and transfer the contents to a beaker, filtering if necessary Dilute to about 200 cc and destroy the excess of H_2O_2 by titration with $KMnO_4$ soln Precipitate the Hg with H_2S, filter thru a weighed Gooch crucible and dry the precipitate in the oven at 105–110 Extract the dried precipitate with CS_2 to remove any precipitated S, again dry, and weigh From the weight of HgS calculate the percentage of metallic Hg, using the factor 0 86219

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VII CAUSTIC POISONS

PHENOL

Method I[1]—*Official*

(Applicable to the determination of phenol in commercial cresols, saponified cresol solns, coal tar dips and disinfectants, and to kerosene solns of phenols except in the presence of salicylates or betanaphthol)

1 REAGENTS

(a) *Dilute nitric acid*—Blow air thru HNO until it is colorless and dilute 1 volume of this acid with 4 volumes of H_2O

(b) *Millon's reagent*—Treat 2 cc of Hg in a 200 cc Erlenmeyer flask with 20 cc of HNO_3 Place the flask under a hood and after the first violent reaction is over shake as much as necessary to effect subdivision of the Hg and maintain action After about 10 min, when the action has practically ceased even in the presence of undissolved Hg add 35 cc of H_2O, and if basic salt separates, add a sufficient quantity of the dilute HNO_3 to dissolve it Add a 10% soln of NaOH dropwise with thoro mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity Then add 5 cc of the dilute HNO_3 and mix well As the soln deteriorates, do not use it later than the day of preparation

(c) *Standard phenol*—Prepare a stock soln by dissolving a weighed quantity of the pure substance (congealing point not lower than 40°) in a sufficient quantity of H_2O to make not less than a 1% soln From this stock soln make a 0 025% soln in additional distilled H_2O (This second soln, which constitutes the final standard should be prepared on the day of use)

(d) *Dilute formaldehyde soln*—Dilute 2 cc of commercial 37% HCHO soln to 100 cc with distilled H_2O

2 APPARATUS

(a) *Nessler cylinders*—50 cc tall form, matched

(b) *Test tubes*—Approximately 180 mm x 20 mm provided with rubber stoppers and marked at 25 cc

(c) *Water bath for heating the test tubes*—A beaker containing a disk of wire gauze raised somewhat from the bottom may be used

3 PREPARATION OF SAMPLE

Commercial Cresol—Weigh by difference about 2 5 g of sample into a 200 cc volumetric flask, dissolve in 10 cc of a 10% NaOH soln, and make to the mark with H_2O

Saponified Cresol Solns, Coal Tar Dips and Disinfectants, Kerosene Solns of Phenols, Etc—Weigh by difference about 5 g (or use 5 cc and calculate the weight from the density of the sample) of sample into a 250 cc volumetric flask and dilute to the mark with H_2O In products consisting largely of kerosene bring the H_2O level to the mark and take aliquots from the aqueous portion only

4 DETERMINATION

Transfer a 5 cc aliquot of the prepared soln to a 200 cc volumetric flask shortly before the determination is to be carried out, dilute to about 50 cc, add 1 drop of methyl orange indicator soln and then dilute HNO_3 (a) until the soln is practically neutral make to volume and shake well

Place 5 cc of the diluted soln in each of 2 of the marked test tubes, and in each of 2 additional test tubes place 5 cc of the standard phenol soln (c) Next flow 5 cc of Millon s reagent (b) down the side of each tube mix and place the tubes in a bath of boiling water, continue the boiling for exactly 30 min , cool immediately and thoroly by immersion in a bath of cold H_2O for at least 10 min , and then add 5 cc of dilute HNO_3 (a) to each tube

Mix well and add 3 cc of dilute HCHO soln, 1 (d) to one of each pair of tubes, make all the tubes to the 25 cc mark with H_2O stopper them, shake well and put aside to stand overnight The next day the contents of the tubes to which HCHO was added will have faded to a yellow while the others will show an orange or red tint

Pipet 20 cc from each of the 2 phenol tubes and transfer to 100 cc volumetric flasks treat each with 5 cc of the dilute HNO_3 (a), make to the mark, and mix The red flask contains the 'phenol standard" and the yellow flask the "phenol blank Transfer these solns to burets Pipet 10 cc of each sample soln into Nessler tubes (The orange or red constitutes the "unknown' and the yellow the "sample blank,' and each Nessler tube must be distinctly marked to avoid confusion) Next add to the "sample blank tube a measured quantity of "phenol standard from its buret and add the same volume of ' phenol blank ' to the "unknown, thoroly agitate (aided by insertion of the rubber stoppers if necessary), and compare the colors When the tubes have in this way been brought to a match each cc of the phenol standard employed is equivalent to 1% of phenol if a portion of sample weighing ex actly 5 g was used, or 2% if exactly 2 5 g was used

NOTE —In using this method the following precautions should be borne in mind A pair of phenol tubes affords sufficient final solns for assaying several unknowns, but all the latter must have accompanied the phenol solns thruout the entire process with identical reagents and treatment If the end point has been inadvertently over run it is possible to work back to it but since mistakes are easy to make in this pro cedure it is better to repeat the comparison on fresh portions from the original tubes Too much delay in matching the tubes must be avoided after the titration has been started, otherwise the excess of HCHO present in the blanks may have time after mixture to affect the intensity of the red color

Millon s reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used

Method II[2]*—Official*

(Applicable to the determination of phenol in the presence of salicylates)

REAGENTS

5 The reagents and solns used are described under 1

DETERMINATION

6 Weigh by difference into a separatory funnel approximately 10 g of sample (or use 10 cc and calculate the weight from the density of the sample) Add 50 cc of kerosene and extract 3 times with 100 cc portions of H_2O Filter the aqueous extracts thru a wet filter into a 500 cc volumetric flask make to volume with distilled H_2O and proceed as directed under 4 beginning with ' Transfer a 5 cc aliquot of this soln to a 200 cc volumetric flask ,"

When the tubes have been brought to a match, each cc of the phenol standard employed is equivalent to 1% of phenol if a portion of the sample weighing exactly 10 g was used

SELECTED REFERENCES

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[2] Ind Eng Chem Anal Ed , 1, 232 (1929)

VII CAUSTIC POISONS

PHENOL

Method I—*Official*

(Applicable to the determination of phenol in commercial cresols saponified cresol soins coal tar dips and disinfectants and to kerosene soins of phenols except in the presence of salicylates or betanaphthol)

1

REAGENTS

(a) *Dilute nitric acid* —Blow air thru HNO until it is colorless and dilute 1 vol ume of this acid with 4 volumes of H_2O

(b) *Millon s reagent* —Treat 2 cc of Hg in a 200 cc Erlenmeyer flask with 20 cc of HNO_3 Place the flask under a hood, and after the first violent reaction is over shake as much as necessary to effect subdivision of the Hg and maintain action After about 10 min when the action has practically ceased even in the presence of undissolved Hg add 35 cc of H_2O and if basic salt separates, add a sufficient quan tity of the dilute HNO_3 to dissolve it Add a 10% soln of NaOH dropwise with thoro mixing until the curdy precipitate that forms after the addition of each drop no longer redissolves but disperses to an evidently permanent turbidity Then add 5 cc of the dilute HNO_3 and mix well As the soln deteriorates, do not use it later than the day of preparation

(c) *Standard phenol* —Prepare a stock soln by dissolving a weighed quantity of the pure substance (congealing point not lower than 40°) in a sufficient quantity of H_2O to make not less than a 1% soln From this stock soln make a 0 025% soln in additional distilled H_2O (This second soln, which constitutes the final standard, should be prepared on the day of use)

(d) *Dilute formaldehyde soln* —Dilute 2 cc of commercial 37% HCHO soln to 100 cc with distilled H_2O

2

APPARATUS

(a) *Nessler cylinders* —50 cc tall form matched

(b) *Test tubes* —Approximately 180 mm x 20 mm, provided with rubber stoppers and marked at 25 cc

(c) *Water bath for heating the test tubes* —A beaker containing a disk of wire gauze raised somewhat from the bottom may be used

3

PREPARATION OF SAMPLE

Commercial Cresol —Weigh by difference about 2 5 g of sample into a 250 cc volu metric flask dissolve in 10 cc of a 10% NaOH soln and make to the mark with H_2O

Saponified Cresol Soins Coal Tar Dips and Disinfectants, Kerosene Soins of Phe nols, Etc —Weigh by difference about 5 g (or use 5 cc and calculate the weight from the density of the sample) of sample into a 250 cc volumetric flask and dilute to the mark with H_2O In products consisting largely of kerosene bring the H_2O level to the mark and take aliquots from the aqueous portion only

4

DETERMINATION

Transfer a 5 cc aliquot of the prepared soln to a 200 cc volumetric flask shortly before the determination is to be carried out dilute to about 50 cc add 1 drop of methyl orange indicator soln and then dilute HNO_3 (a) until the soln is practically neutral make to volume and shake well

Place 5 cc of the diluted soln in each of 2 of the marked test tubes, and in each of 2 additional test tubes place 5 cc of the standard phenol soln (c) Next flow 5 cc of Millon s reagent (b) down the side of each tube mix, and place the tubes in a bath of boiling water continue the boiling for exactly 30 min , cool immediately and thoroly by immersion in a bath of cold H_2O for at least 10 min , and then add 5 cc of dilute HNO_3 (a) to each tube

Mix well and add 3 cc of dilute HCHO soln, 1 (d), to one of each pair of tubes, make all the tubes to the 25 cc mark with H_2O, stopper them, shake well and put aside to stand overnight The next day the contents of the tubes to which HCHO was added will have faded to a yellow while the others will show an orange or red tint

Pipet 20 cc from each of the 2 phenol tubes and transfer to 100 cc volumetric flasks treat each with 5 cc of the dilute HNO_3 (a), make to the mark and mix The red flask contains the 'phenol standard" and the yellow flask the phenol blank , Transfer these solns to burets Pipet 10 cc of each sample soln into Nessler tubes , (The orange or red constitutes the "unknown and the yellow the "sample blank , and each Nessler tube must be distinctly marked to avoid confusion) Next add to the "sample blank tube a measured quantity of "phenol standard ' from its buret and add the same volume of ' phenol blank ' to the "unknown," thoroly agitate (aded by insertion of the rubber stoppers if necessary) and compare the colors When the tubes have in this way been brought to a match each cc of the phenol standard employed is equivalent to 1% of phenol if a portion of sample weighing exactly 5 g was used or 2% if exactly 2 5 g was used

NOTE —In using this method the following precautions should be borne in mind A pair of phenol tubes affords sufficient final solns for assaying several unknowns, but all the latter must have accompanied the phenol solns thruout the entire process with identical reagents and treatment If the end point has been inadvertently over run it is possible to work back to it but since mistakes are easy to make in this procedure it is better to repeat the comparison on fresh portions from the original tubes Too much delay in matching the tubes must be avoided after the titration has been started otherwise the excess of HCHO present in the blanks may have time after mixture to affect the intensity of the red color

Millon s reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used

Method II[2]*—Official*

(Applicable to the determination of phenol in the presence of salicylates)

REAGENTS

5 The reagents and solns used are described under 1

DETERMINATION

6 Weigh by difference into a separatory funnel approximately 10 g of sample (or use 10 cc and calculate the weight from the density of the sample) Add 50 cc of kerosene and extract 3 times with 100 cc portions of H_2O Filter the aqueous extracts thru a wet filter into a 500 cc volumetric flask make to volume with distilled H_2O, and proceed as directed under 4, beginning with 'Transfer a 5 cc aliquot of this soln to a 200 cc volumetric flask '

When the tubes have been brought to a match, each cc of the phenol standard employed is equivalent to 1% of phenol if a portion of the sample weighing exactly 10 g was used

SELECTED REFERENCES

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[2] Ind Eng Chem , Anal Ed , 1, 232 (1929)

VIII NAVAL STORES

ROSIN

TOLUOL-INSOLUBLE MATERIAL IN ROSIN[1]—TENTATIVE

1 PREPARATION OF SAMPLE

(1) If the sample is less than 200 g, immediately before the determination is made powder it to pass a standard 10 mesh sieve, mix thoroly, and place in a wide mouthed bottle of such size that the sample completely fills it

(2) If the sample is more than 200 g, crush it to pass a ¼ inch sieve, mix, quarter down to about 200 g, and treat as described in (1)

2 PROCEDURE

Place 50 g of the freshly-powdered sample in a 300 cc beaker, add 150 cc of toluol free from H_2O and non volatile residue, and dissolve the sample with the aid of heat and occasional shaking When the soln is apparently complete (no particles of rosin visible), filter at once thru a 25 cc porcelain Gooch crucible which has been previously prepared with a mat of pure, well washed asbestos (such as is used for the determination of $BaSO_4$) and which has been finally washed thoroly with the solvent used, dried in a boiling water oven for 30 min , cooled in a desiccator, and weighed *If the rosin filtrate is not clear, return it thru the Gooch crucible until it* is clear, finally washing the residue and the outside of the crucible free from rosin with additional hot solvent Dry the crucible and contents to constant weight at 105–110° in an oven (1 hour usually suffices), cool in a desiccator weigh, and calcu late the percentage of toluol insoluble

TURPENTINE OIL[2] (SPIRITS OF TURPENTINE)

3 COLOR—TENTATIVE

Place a 200 cc flat bottomed colorimeter tube graduated in mm and filled to a depth of 40–50 mm with the turpentine in a colorimeter and on or under it place a No 2 yellow Lovibond glass Over or under a second graduated tube in the colori meter, place a No 1 yellow Lovibond glass and run into it the same turpentine until the color matches as nearly as possible the color in the first tube Read the difference in depth of the turpentine in the 2 tubes If this difference is 50 mm or more, the turpentine is "standard if it is 150 mm or more, the turpentine is "waterwhite", and if the difference is from 25 to 49 9 mm the turpentine is "off one shade '

4 SPECIFIC GRAVITY

Determine the specific gravity at 15 5/15 5 by any convenient method that is accurate within 2 points in the fourth place If the determination is made at any other temp , correct the reading by adding thereto or subtracting therefrom 0 00082 for each degree that the temp at which the determination is made is re spectively above or below 15 5°

5 REFRACTIVE INDEX—TENTATIVE

Determine the refractive index at any convenient temp , but preferably at 20° If determined at other than 20°, calculate the result to 20° by adding or subtracting

the correction factor 0 00045 for each degree that the temp of the determination is above or below 20 , respectively

DISTILLATION—TENTATIVE

6 APPARATUS

(a) *Flask* —Use an Engler flask having the following dimensions Diameter of bulb, 6 5 cm, cylindrical neck 15 cm long 1 6 cm internal diameter, side or vapor tube, 10 cm long 0 6 cm external diameter, attached to neck at an angle of 75°, so that when the flask contains its charge of 100 cc of oil the surface of the oil shall be 9 cm below the bottom of the junction of the side tube and neck

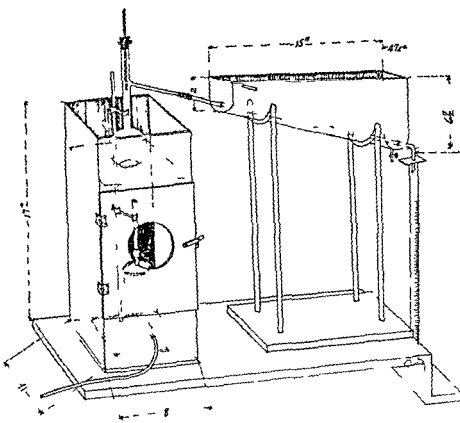

FIG 9—APPARATUS FOR THE DISTILLATION OF TURPENTINE OIL OVER OPEN FLAME

Support the flask on a plate of asbestos 20 cm square, having an opening 4 cm in diameter in its center and heat with an open flame or, support the flask in a metal cup, 15-20 cm in diameter containing high boiling mineral oil or glycerol and fitted with a concave cover having in the center a circular opening 5½ 6 cm in diameter (Fig 10) Surround the flask and burner with a shield to prevent fluctuation in temp in the neck of the flask

(b) *Condenser* —(1) Use the form[a] illustrated in Fig 9, which consists of thin-walled brass condenser tubing (No 20 Stubbs gage seamless) ½ inch inside diameter and 22 inches long placed at an angle of 75° in a metal cooling bath of the size and dimensions shown in Fig 9 The lower end of the condenser is cut off at an acute angle and curved down for a length of 3 inches so as to project at least ½ inch into the receiving cylinder, or (2) use a straight glass condenser 22 inches long, having 16

inches in contact with the cooling water and fitted with an adapter, the small end of which, cut off at an acute angle, is long enough to extend a short distance into the receiving cylinder as illustrated in Fig 10

(c) *Thermometer* — Use an accurate thermometer of the Anschutz type, conforming to the following specifications Graduated from 145 to 200° in 0 2° intervals Length, bottom of thermometer to 175° mark, not more than 8 nor less than 6 5 cm top of bulb to 145° mark, not less than 1 5 cm, from 145 to 175° mark not more than 6 cm The graduation marks and the numbering shall be clear-cut and distinct The error at any point on the scale shall not exceed ±0 5° when tested for total immersion of the Hg column

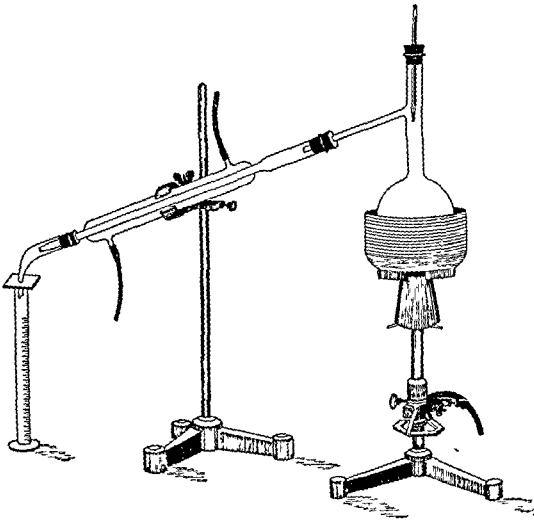

FIG 10 —APPARATUS FOR THE DISTILLATION OF TURPENTINE OIL OVER BATH

(d) *Receiving cylinder* —Use an accurately graduated 50–100 cc cylinder The so-called normal or precision cylinder of 50 cc capacity, having an internal diameter of 1 5 cm and graduated in 0 2 cc is preferred If a cylinder with larger inside diameter is used, place over the top a pasteboard cover having an opening for the condenser tube

7 DETERMINATION

Place 100 cc of the turpentine and several small pieces of pumice (or glass) in the distilling flask Fit the thermometer so that the top of the Hg bulb is level with the

bottom of the side tube and the 175° mark is below the cork Place the flask in position on the asbestos board or oil bath and connect with the condenser Apply the heat cautiously at first and when distillation begins so regulate that the turpentine distils at the rate of not less than 4 nor more than 5 cc per min (approximately 2 drops per second) The initial boiling point is the thermometer reading at the instant when the first drop falls from the end of the condenser Discontinue distillation when the temp reaches 170 0° or the equivalent thereof depending on the atmospheric pressure, as determined under 8 let the condenser drain, and read the percentage distilled The percentage distilled below successive selected temps and the temp at which each successive 10 cc distils may also be determined if desired the necessary correction of the temp being made for variations in atmospheric pressure

CORRECTION FOR VARIATION IN ATMOSPHERIC PRESSURE

8 The distilling temp of turpentine is affected 0 057° for each millimeter variation in barometric pressure If the barometer reading after correcting to 0 is above or below the normal 760 mm the turpentine will distil at a higher or lower temp , respectively, than at normal pressure Therefore, for each mm that the corrected barometer reading is above 760 mm correct the initial boiling point reading by minus (−) 0 0 57 and for each mm that the corrected barometer reading is below 760 mm, correct the initial boiling point reading by plus (+) 0 0 57° Also correct the final temp observation point (170) in the same way by adding thereto 0 057° for each mm of pressure above 760 mm or subtracting therefrom 0 057 for each mm of pressure below 760 mm, as may be required The actual temp at which distillation is stopped must be that equivalent to 170 at 760 mm

MINERAL OIL IN TURPENTINE—OFFICIAL

Fuming Sulfuric Acid Method

REAGENTS

9 *Fuming 35 N sulfuric acid* —Mix concentrated sulfuric acid with sufficient liquid fuming H_2SO_4 to obtain a mixture containing slightly more than 82 38% total SO_3 If the fuming acid contains 50% excess SO_3 about 100 g of fuming acid to 140 g of concentrated acid will be approximately the correct ratio Determine the exact strength of the mixture and also of a reserve supply of concentrated acid as follows Weigh a quantity of the acid in a weighing bulb or pipet having a capillary tube at the lower end and a stopcock at the upper end and fitted with a Pt wire for suspending on the balance Fill the bulb by slight suction and empty the lower end of the capillary by closing the stopcock simultaneously with the withdrawal of the capillary from the acid wiping off first with a moist and then with a dry cloth Allow the acid to flow down the sides of the neck of a volumetric flask into cold H_2O (If a flask approximately 100 times the volume of the weighing pipet is used the result ant soln will be near half normal) Wash all traces of acid into the flask taking precautions to prevent loss of SO_3 fumes Make to volume and titrate from a buret against standard alkali using the indicator with which the alkali was standardized Calculate the SO_3 content of both acids and add sufficient concentrated acid to the fuming mixture to bring it to 82 38°c After mixing determine the strength of this fuming mixture as before The SO_3 content of this acid must not vary more than +0 05°c or −0 05°c from 82 38°c The acid must be carefully protected against absorption of moisture from the air

DETERMINATION

10 Place 20 cc of the 35 N fuming H_2SO_4 in a graduated narrow necked Babcock flask stopper and place in ice H O to cool Add slowly from a pipet 5 cc of the tur

pentine, gently shaking or rotating the flask and keeping the temp at about 60–65 by continued immersion in ice H_2O When the mixture no longer develops heat on shaking, agitate thoroly by vigorously shaking for about ½ min Place the flask in a water bath and heat at 60–65° for 10 min , keeping the contents of the flask thoroly mixed by shaking vigorously not less than 6 times during the heating period (CAUTION If the shaking is too vigorous at first, there is danger of the escaping SO_2 forcing some of the mixture up over the mouth of the flask) Cool to room temp and fill the flask with concentrated H_2SO_4 until the surface rises well into the graduated neck Centrifugalize for 5 min at 1200 r p m , or for 10 min at 900 r p m , or allow to stand, lightly stoppered, for 12 hours Read the volume of unpolymerized residue (middle of meniscus), calculate the percentage, record its consistency and color, and determine its refractive index at 20°

Pure gum spirits of turpentine by this method gives less than 2 0% residue, which has a straw or darker color, viscous consistency, and a refractive index of not less than 1 500 A limpid colorless residue with a refractive index of less than 1,500 indicates the presence of mineral oil The unpolymerized residue from an adulterated oil represents from 60–80% of the total quantity of adulterant present

11 *Sulfuric-Fuming Nitric Acid Method*[5]*—Tentative*

Place 50 cc of the turpentine in a 300 cc Kjeldahl or other long necked flask, cool in ice H O, and add slowly with constant agitation 25 cc of H_2SO_4 Shake well to obtain complete reaction, keeping the flask cool When the reaction is complete, cool thoroly and add 25 cc of H_2O Distil the polymerized mixture in a current of steam, collecting 300 cc of total distillate Separate the oil from the aqueous portions

Place a volume of fuming (sp gr 1 5) HNO_3 equal to 3 times the volume of the oil in a 200–250 cc separatory funnel and cool in ice H_2O Add the oil cautiously dropwise, shaking carefully and keeping the mixture cool After all the oil has been added, allow the funnel to stand quietly, very lightly stoppered, about 30 seconds, until the oil has had a chance to come to the surface Then draw off the acid and wash the remaining oil once with a little fuming HNO_3 once with HNO_3, and finally several times with H_2O Measure the volume of the oil, record its consistency and color, and determine its refractive index at 20° Pure gum spirits of turpentine gives less than 0 5% residue by this method

SELECTED REFERENCES

[1] J Assoc Official Agr Chem , 13, 48 (1930)
[2] U S Dept Agr Bur Chem Bull 898, U S Bur Standards Circ , 86
[3] Adopted by American Society for Testing Materials
[4] Chem Ztg , 30, 631 (1906), U S Dept Agr Bur Chem Circ , 85, J Assoc Official Agr Chem , 6, 465 (1923)
[5] J Ind Eng Chem , 1909 1, 27 (1909), J Assoc Official Agr Chem , 5, 547 (1922), 9, 55 (1926)

IX PAINTS—TENTATIVE

WHITE LINSEED OIL PAINTS[1]

PRELIMINARY PROCEDURE

1 On receipt of a sample make a record of the label noting especially the brand the name of the manufacturer and any statement as to composition and net con tents Weigh the unbroken package, open note odor and condition of the contents pour into a clean container and mix thoroly by pouring from one container to the other finally leaving the well mixed sample in the second container, which shall be tightly closed The well mixed sample is used at once for the determinations de scribed under 'Methods The original can and cover may be cleaned with gasoline wiped dry, and then weighed This weight subtracted from the original weight will give the net weight of the contents If desired the specific gravity of the paint may be determined and the weight per gallon calculated and the volume of paint and the capacity of the container may be measured

REAGENTS

2 (a) *Extraction mixture* —10 volumes ethyl ether 6 volumes benzol 4 volumes methyl alcohol, and 1 volume acetone

(b) *Aqueous sodium hydroxide soln* —Dissolve 100 g of NaOH and dilute to 100 cc

(c) *Alcoholic sodium hydroxide soln* —Dissolve NaOH in 95% ethyl alcohol in the proportion of about 22 g per 1000 cc Let stand in a stoppered bottle Decant the clear liquid into another bottle and keep well stoppered This soln should be color less or only slightly yellow when used It will keep colorless longer if the alcohol is previously treated with NaOH (about 50 g to 1000 cc) kept at about 50° for 15 days and then distilled

(d) *Iodine soln* —Dissolve I in glacial acetic acid that has a melting point of 14.7–15° and is free from reducing impurities in a proportion that 13 g of I will be present in 1000 cc of soln The preparation of the I monochloride soln presents no great difficulty but it should be done with care and accuracy in order to obtain satisfactory results There should be in the soln no sensible excess either of I or more particularly of Cl over that required to form the monochloride This condition is most satisfactorily attained by dissolving in the whole of the acetic acid to be used the requisite quantity of I using a gentle heat to assist the soln if it is found necessary Set aside a small portion of this soln while pure and pass dry Cl into the remainder until the halogen content of the soln is doubled Ordinarily it will be found that by passing the Cl into the main part of the soln until the characteristic color of free I has just been discharged there will be a slight excess of Cl which is corrected by the addition of the requisite amount of the unchlorinated portion until all free Cl has been destroyed A slight excess of I does little or no harm but excess of Cl must be avoided

(e) *Standard sodium thiosulfate soln* —Dissolve pure $Na_2S_2O_3$ in distilled H_2O (well boiled to free it from CO_2) in the proportion of 24.83 g of $Na_2S_2O_3 \cdot 5H_2O$ to 1000 cc of the soln It is best to let this soln stand for about 2 weeks before standardizing Standardize with pure resublimed I KI by iodate or pure KIO_3 (see Treadwell Hall

[1] Methods (D 215–) of the American Society for Testing Materials and adopted tentatively at the 1930 meeting of the A S T M These methods have been edited to conform in part to the style of the publication but otherwise they are as published in the 1931 Supplement to Book of A S T M Standards Under the standardization procedure of the A S T M these methods are under the jurisdiction of the A S T M Committee D 1 on Preservative Coatings for Structural Materials

Analytical Chemistry, Vol 2) This soln will be approximately 0 1 *N*, and it is best to leave it as it is after determining its exact I value, rather than to attempt to ad just it to exactly 0 1 *N* Preserve in a stock bottle provided with a guard tube filled with soda lime

(f) *Starch soln* —Stir up 2–3 g of potato starch or 5 g of soluble starch with 100 cc of 1% salicylic acid soln, add 300–400 cc of boiling H_2O, and boil the mixture until the starch is practically dissolved then dilute to 1 liter

(g) *Potassium iodide soln* —Dissolve 150 g of KI free from iodate in H_2O and di lute to 1000 cc

(h) *Acid ammonium acetate soln* —Mix 150 cc of 80% acetic acid 100 cc of H_2O and 95 cc of NH_4OH (sp gr 0 90)

(i) *Ammonium polysulfide* —Pass H_2S gas into 200 cc of NH_4OH in a bottle im mersed in running H_2O or in iced H_2O until the gas is no longer absorbed, then add 200 cc of NH_4OH and dilute with H_2O to 1000 cc Digest this soln with 25 g of flowers of S for several hours and filter

(j) *"Lead acid"* —Mix 300 cc of H_2SO and 1800 cc of H_2O Dissolve 1 g of c p Pb acetate in 300 cc of H_2O and add this to the hot soln, stirring meanwhile Let stand at least 24 hours and siphon thru a thick asbestos filter

(k) *Potassium permanganate soln* —Dissolve 3 2 g of $KMnO_4$ in 1 liter of H_2O, let stand 8–14 days siphon off the clear soln (or filter thru an asbestos filter), and standardize as follows In a 400 cc beaker dissolve 0 25–0 30 g (accurately weighed) of Bureau of Standards Na oxalate in 250 cc of hot H O (80–90) and add 15 cc of sulfuric acid (1+1) Titrate at once with the $KMnO_4$ soln, stirring vigorously and continuously The $KMnO_4$ must not be added more rapidly than 10–15 cc per min and the last 0 5–1 cc must be added dropwise with particular care to allow each drop to be fully decolorized before the next is introduced The temp of the soln should not be below 60° by the time the end point is reached (Too rapid cooling may be prevented by allowing the beaker to stand on a small asbestos covered hot plate during the titration The use of a small thermometer as a stirring rod is most con venient) The weight of Na oxalate used multiplied by 0 833 gives its Fe equivalent Keep the $KMnO_4$ soln in a glass stoppered bottle painted black to keep out light

The Fe value of the $KMnO_4$ multiplied by 1 076 theoretically equals its Sb equiv alent However, for use in determining Sb the $KMnO_4$ is best standardized as fol lows To 0 25 g of pure metallic Sb in a 500 cc Pyrex Erlenmeyer flask, add 12–15 cc of H_2SO_4 and 10–12 g of K_2SO_4, heat until all the Sb is dissolved cool, dilute to 250 cc with H_2O add 20 cc of HCl, cool to 10–15°, and titrate with the $KMnO_4$ soln until a faint pink color is obtained For special work after digesting dilute to 100 c with H_2O add 1–2 g of Na_2SO_3, and boil until all the SO is expelled This is shown when no blue color is obtained with starch iodate paper (r), the volume will be re duced about one half Dilute to 250 cc with H_2O, add 20 cc of HCl (sp gr 1 19), and complete as described

(l) *Standard potassium ferrocyanide* —Dissolve 22 g of the pure salt in H_2O and dilute to 1000 cc To standardize transfer about 0 2 g (accurately weighed) of pure metallic Zn or freshly ignited pure ZnO to a 400 cc beaker Dissolve in 10 cc of HCl and 20 cc of H_2O Drop in a small piece of litmus paper, add NH_4OH until slightly alkaline, then add HCl until just acid, and then 3 cc of HCl Dilute to about 250 cc with hot H_2O and heat nearly to boiling Run in the ferrocyanide soln slowly from a buret with constant stirring until a drop tested on a white porcelain plate with a drop of the uranyl indicator shows a brown tinge after standing 1 min A blank should be run with the same amounts of reagents and H_2O as in the standardization The amount of ferrocyanide soln required for the blank should be subtracted from

the amounts used in standardization and in titration of the sample The standardization must be made under the same conditions of temp , volume, and acidity as obtained when the sample is titrated

(m) *Uranyl indicator for zinc titration* —A 5% soln of uranyl nitrate in H_2O or a 5% soln of uranyl acetate in H_2O made slightly acid with acetic acid

(n) *Alkaline lead nitrate soln* —Into 100 cc of KOH soln (56 g in 140 cc of H_2O) pour a saturated soln of $Pb(NO_3)_2$ (250 g in 500 cc of H_2O) until the precipitate ceases to redissolve, stirring constantly while mixing Let settle filter thru asbestos, and dilute the clear filtrate with an equal volume of H_2O About 3 volumes of the $Pb(NO_3)_2$ soln will be required for 1 of the KOH

(o) *Ammoniacal cadmium chloride or zinc sulfate soln* —Dissolve 8 g of $CdCl_2$ in 200 cc of H_2O and add 200 cc of NH_4OH (sp gr 0 90) or dissolve 50 g of $Zn(SO_4)_2$ in 270 cc of H_2O and 230 cc of NH_4OH (sp gr 0 90)

(p) *Standard potassium iodate soln* —Dissolve 3 6 g of KIO_3 and 39 g of KI in 1000 cc of H_2O For general work the theoretical sulfur titer of this soln should be used, for special work, the soln may be standardized against like material, such as a lithopone of known sulfide S content The theoretical titer is based on standard $Na_2C_2O_4$ and is obtained as follows To 300 cc of H_2O in a 600 cc flask, preferably glass stoppered, add 10 cc of HCl (sp gr 1 19) and 1 g of KI Cool and add 10 cc of 0 1 *N* $KMnO_4$ soln which has been standardized against $Na_2C_2O_4$ Swirl gently stopper and let stand for 5 min Titrate the liberated I with standard $Na_2S_2O_3$ soln until the color fades Then add 10 cc of starch soln and continue the titration until the blue color is destroyed Repeat the titration, substituting 10 cc of the iodate soln for the $KMnO_4$ soln Calculate the normality of the iodate soln

(q) *Starch indicator for sulfur titration* —(1) To 1000 cc of boiling H_2O add a cold suspension of 6 g of starch in 100 cc of H_2O and boil vigorously for 5 min Cool the soln, add 6 g of $ZnCl_2$ dissolved in 50 cc of cold H_2O thoroly mix and set aside for 24 hours Decant the clear supernatant liquid into a suitable container add 3 g of KI and mix thoroly (2, Optional) Prepare an emulsion of 6 g of soluble starch in 25 cc of H_2O, add a soln of 1 g of NaOH in 10 cc of H O, and stir the soln until it gelatinizes Dilute to 1000 cc with H_2O add 3 g of KI and mix thoroly

(r) *Starch iodate paper* —Impregnate filter paper with a soln obtained by heating 2 g of starch with 100 cc of H_2O and after soln adding 0 2 g of KIO_3 dissolved in 5 cc of H_2O

(s) *Standard iodine soln for SO_2* —Place 15–20 g of pure KI in a liter volumetric flask, dissolve in as little H_2O as possible and then add about 6 4 g of resublimed I Shake until all the I is dissolved dilute to the mark with H_2O, and mix This soln is approximately 0 05 *N* and is standardized against 0 05 *N* $Na_2S_2O_3$ to obtain its true normality

(t) *Standard sodium thiosulfate soln for SO_2* —Prepare and standardize as described in (e) using 12 42 g of $Na_2S_2O_3 \cdot 5H_2O$ or the 0 1 *N* soln may be diluted with an equal volume of cold CO_2-free H_2O

(u) *Ferric sulfate soln for titanium* —Prepare a soln containing 2% of Fe as ferric sulfate as follows Dissolve 20 g of pure Fe or plain C steel in a slight excess of HCl oxidize with HNO_3 add about 80 cc of H_2SO_4 and heat until fumes of the latter are evolved Cool dilute with H_2O to 1000 cc digest on a steam bath until sulfates are dissolved and filter if necessary Add 0 1 *N* $KMnO_4$ soln until a faint pink color persists for 5 min (to oxidize any ferrous Fe that may be present) Ferric ammonium sulfate may be used also

(v) *Standard ferric sulfate soln for colorimetric determination of iron* —Determine the strength of the ferric soln for the TiO_2 determination in terms of Fe and dilute

a portion of this soln until one is obtained of the strength 1 cc = 0 00001 g of Fe

(w) *Potassium thiocyanate indicator*—Prepare a 2% soln of the pure salt in H_2O

METHODS

3 WATER[1]

Mix 100 g of the paint in a 250 cc flask with 75 cc of toluene Place the flask in an oil bath, connect with condenser, apply heat to the bath, and distil until about 50 cc of distillate has been collected in a graduate The temp in the flask should be then 105–110° The number of cc of H_2O collected under the toluene in the receiver is the percentage of H_2O in the paint

4 VOLATILE THINNER

Weigh accurately 3–5 g of the paint into a tared flat bottomed dish about 8 cm in diameter, spreading the paint over the bottom Heat at 105–110° for 1 hour, cool, and weigh Calculate the loss in weight as percentage of H O and volatile thinner subtract from this the percentage of H_2O (3), and report the remainder as volatile thinner

5 NATURE OF THE THINNER

Transfer about 150 g of the paint to a 500 cc flask fitted with a 2 hole cork stopper carrying a spray trap connected with a vertical condenser Thru the other hole in the stopper pass an influx tube for steam (This tube should dip below the surface of the paint) Heat the flask in an oil bath or an air bath at 100 and pass thru it a current of steam, with the steam still passing thru, raise the temperature of the bath to 130° Catch the distillate in a small separatory funnel and continue distillation until 300 cc of H_2O has been condensed Portions of this H_2O may be drawn from the cock of the separatory funnel from time to time but care must be taken not to draw out any of the volatile thinner Let the distillate stand until it separates into 2 layers, then draw off the H_2O, and filter the volatile thinner thru a dry filter paper into a dry flask If the thinner is apparently turpentine, examine the distillate as directed[2] in Chap VIII If the thinner is a mixture of turpentine and mineral spirits, an approximate determination of the amount of turpentine may be made by the polymerization test specified for under turpentine It should be noted that turpentine is slightly soluble in H_2O (about 0 3–0 4 cc per 100 cc of H_2O)

To test for benzol, add a few drops of the distillate to a small quantity of a mixture of HNO_3 and H_2SO_4, and heat cautiously The characteristic odor of nitrobenzol will be noted if benzol is present

If the thinner is apparently all mineral spirits, no further examination is necessary

6 PERCENTAGE OF PIGMENT

Strain a portion of the well mixed sample thru a No 80 sieve[3] to remove any skins and weigh accurately about 15 g of the strained paint in a weighed centrifuge tube Add 20–30 cc of "extraction mixture' [Reagent 2(a)], mix thoroly with a glass rod wash the rod with more of the extraction mixture, and add enough of the reagent to make a total of 60 cc in the tube Place the tube in the container of a centrifuge surround the tube with H_2O, and counterbalance the container of the opposite arm with a similar tube or a tube with H_2O Whirl at a moderate speed until well

[1] A convenient apparatus for this determination is shown in Fig 1 (b) of the Standard Method of Test for Water in Petroleum Products and Other Bituminous Materials (A S T M Designation D 95) of the American Society for Testing Materials (1927 Supplement to Book of A S T M Standards p 149)

[2] 1927 Book of A S T M Standards Part II p 203 or 1930 ed Part II p 337

[3] Sieve opening 1 7 mm wire diameter 119 mm

9 TEST FOR MINERAL OIL AND OTHER UNSAPONIFIABLE MATTER

Place 10 drops of the fatty acids (8), in a 50 cc test tube, add 5 cc of alcoholic soda [Reagent 2(c)], boil vigorously for 5 min , add 40 cc of H_2O, and mix A clear soln indicates that not more than traces of unsaponifiable matter are present

10 IODINE NUMBER OF FATTY ACIDS[1]

Place a small quantity of the fatty acids (8), in a small weighing buret or beaker Weigh accurately Transfer by dropping about 0 15 g (0 10–0 20 g) into a 500 cc bottle having a well ground-glass stopper, or an Erlenmeyer flask having a specially flanged neck for the I test Reweigh the buret or beaker and determine the amount of sample used (If desired the sample may be weighed in a small wide mouthed vial and the vial containing the weighed sample placed in the bottle or flask) Add 10 cc of $CHCl_3$ Whirl the bottle or flask to dissolve the sample Add 10 cc of $CHCl_3$ to 2 empty bottles or flasks like that used for the sample Add to each bottle or flask 25 cc of the Wijs soln [Reagent 2(d)] and let stand with occasional shaking for 1 hour in a dark place at a temp of from 21 to 23° Add 10 cc of the 15% KI soln and 100 cc of H_2O, and titrate with standard $Na_2S_2O_3$ soln [Reagent 2(e)] using starch as indicator The titrations on the 2 blank tests should agree within 0 1 cc From the difference between the average of the blank titrations and the titration on the sample and the I value of the thiosulfate soln, calculate the I number of the sample tested (I number is given in centigrams of I to 1 g of sample)

11 ROSIN

I Liebermann-Storch Test[2]

To about 1 g of the fatty acids add 15 cc of acetic anhydride and shake until soln is complete Pour a few drops of this soln on a white porcelain plate (a crucible cover serves well) and add a drop of H_2SO_4 (sp gr 1 53) A fugitive violet color indicates rosin

II Halphen Hicks Test[3]

Test the fatty acids with the Halphen Hicks reagent as follows

Soln A —Dissolve 1 part by volume of phenol in 2 parts by volume of CCl_4

Soln B —Dissolve 1 part by volume of Br in 4 parts by volume of CCl_4

Add 1–2 cc of Soln A to about 1 g of the fatty acids and pour this mixture into a cavity of an ordinary porcelain color reaction plate until it just fills the depression Immediately fill an adjacent cavity with Soln B Cover the plate with an inverted watch glass and note the color, if any, produced in the former soln by the action of the Br vapors from Soln B A decided purple or deep indigo blue color is an indication of the presence of rosin

PIGMENT

12 *A Qualitative Analysis*

A complete qualitative analysis, following the well established methods should be made and the quantitative scheme modified as required Add acetic acid slowly to the pigment until all carbonate is decomposed (noting whether any H_2S is evolved)

[1] If appreciable amounts of rosin or of unsaponifiable matter are found to be absent in the vehicle of a paint the I number of the fatty acids gives the best indication (tho not proof) of the presence of linseed oil An I number of less than 170 (Wijs) for the fatty acids is an indication that the non volatile vehicle was not pure linseed oil.

[2] Lewkowitsch Chemical Technology and Analysis of Oils Fats and Waxes Vol 1 p 693 (1921)

[3] *J Ind Eng Chem* 3 86 (1911)

then add a large excess of acid NH_4 acetate soln [Reagent 2(h)], boil, filter, and test the filtrate for metals other than Pb and Zn (especially Ca and Ba) The absence of Ca in this filtrate indicates that the extending pigments contain no $CaCO_3$ or $CaSO_4$ the absence of Ba indicates that the extending pigments contain no $BaCO_3$.[1] Wash the matter insoluble in acid NH_4 acetate soln with another portion of this soln, and finally with hot H_2O This insoluble matter is dried, ignited, and tested for siliceous matter $BaSO_4$, and Ti compounds To test for the Ti compounds place a small amount of the insoluble matter, or of the original sample (about 0 5 g), in a 250 cc Pyrex glass beaker add 20 cc of concentrated H_2SO_4 and 7–8 g of $(NH_4)_2SO_4$ Mix well, and boil for a few min A residue denotes the presence of SiO_2, siliceous matter, or $BaSO_4$ Cool the soln, dilute with 100 cc of H_2O heat to boiling settle, filter, wash with hot 5% H_2SO_4 until free from Ti The residue may be tested for Pb Ba, and SiO_2 Add H O 2 to a small portion of the filtrate a clear yellow orange color indicates the presence of Ti Boil another portion of the filtrate with metallic tin or Zn A pale blue to violet coloration indicates Ti Treat another portion (about 1 g) of the pig ment with 20 cc of HCl (1+1) and note whether any H_2S is evolved boil the soln for about 5 min add about 25 cc of hot H_2O, filter and wash with hot H_2O Render a small portion of the filtrate alkaline with NH_4OH, acidify with HCl, and add a little $BaCl_2$ soln, a white precipitate ($BaSO_4$) indicates the presence of a soluble sulfate To another portion of the filtrate add a little H_2SO_4 (a white precipitate indicates the presence of Pb, soluble Ba or both (some $CaSO_4$ may also separate), filter, wash to remove free acid and treat the precipitate with a few drops of KI soln (the forma tion of yellow PbI_2 indicates the presence of Pb) The white precipitate may also be treated with H_2S water, the formation of black PbS indicates the presence of lead To another portion of the original filtrate add NH_4OH until alkaline, render slightly acid with acetic acid heat to boiling and add a little $K_2Cr_2O_7$ soln, a yellow or orange yellow precipitate indicates the presence of Pb soluble Ba or both To an other portion of the original filtrate add a few drops of $K_4Fe(CN)_6$ soln A white precipitate with a bluish tinge indicates the presence of Zn Pass into the remaining portion of the original filtrate a current of H_2S for 5–10 min add an equal volume of H O and pass H_2S into the soln for about 5 min filter wash with H_2S water then digest the precipitate with NH_4 polysulfide filter, acidify the filtrate with HCl, and warm, the presence of Sb is indicated by the separation of an orange colored precip itate The filtrate from the H_2S precipitate may be tested for Ba Ca and Mg in the usual manner

B *Quantitative Analysis*

13 SPECIFIC GRAVITY

14 If the determination of specific gravity of the pigment is required determine ac cording to the Standard Methods of Test for Specific Gravity of Pigments (Serial Designation D 153) of the American Society for Testing Materials.[2]

SINGLE PIGMENTS

15 BASIC CARBONATE OF LEAD

(a) *Total Lead (Gravimetric)*.—Dissolve 1 g in 20 cc of HNO_3 (1+1) in a covered beaker heating till all CO_2 is expelled wash off cover, add 20 cc of H_2SO_4 (1+1) and

[1] If the original sample contained $BaCO_3$ and $PbSO_4$, $CaSO_4$ or still more soluble sulfate of Ba will form with the soluble sulfate a precipitate of $BaSO_4$ which will be determined as insoluble matter. If the sample contains neither $SrCO_3$ nor $SrSO_4$ some $SrSO_4$ may be counted as $BaSO_4$ some $SrCO_3$ may be counted as CaO and some may be counted as $CaSO_4$. Thus element is not separated as it probably will not be encountered or will be present as an impurity in the Ba and Ca compounds.

[2] 1917 A S T M Standards Vol II p 777 or 1930 A S T M Standards Part p 310

evaporate to fumes of SO_2, cool, add about 150 cc of H_2O and 150 cc of alcohol, let stand in *cold* H_2O 1 hour, filter on a Gooch crucible, wash with 95% alcohol, dry at 110°, and weigh $PbSO_4$, calculate to PbO or to basic carbonate[1] Instead of determining the Pb as sulfate, the sample may be dissolved by boiling with acetic acid, then dilute to about 200 cc with H_2O, make alkaline with NH_4OH, then acid with acetic acid, heat to boiling and add 10–15 cc of a 10% soln of $K_2Cr_2O_7$, heat till the yellow precipitate assumes an orange color Let settle and filter on a Gooch crucible, washing by decantation with hot H_2O till the washings are colorless, finally transferring all the precipitate Wash with 95% alcohol and then with ether, dry at 110° and weigh $PbCrO_4$ (Any insoluble matter should be filtered out before precipitating the Pb)

(b) *Total Lead (Volumetric)* —Dissolve 0 5 g of sample in 10 cc of HCl, boil till soln is effected, cool, dilute to 40 cc, neutralize with NH_4OH Add acetic acid until distinctly acid, dilute to 200 cc with hot H_2O, boil and titrate with NH_4 molybdate as follows

Dissolve 4 25 g of NH_4 molybdate in H_2O and make up to 1 liter To standardize this soln, dissolve about 0 2 g of pure Pb foil in HNO_3 (pure PbO or $PbSO_4$ may also be used), evaporate nearly to dryness, add 30 cc of H_2O, then 5 cc H_2SO_4 (sp gr 1 84), cool, and filter Drop filter with $PbSO_4$ into a flask, add 10 cc of HCl, boil till completely disintegrated add 15 cc of HCl 25 cc of H O, and NH_4OH till alkaline Acidify with acetic acid dilute to 200 cc with hot H_2O and boil Titrate using an outside indicator of 1 part of tannic acid in 300 parts of H_2O

It should be noted that when Ca is present, it forms a more or less insoluble molybdate, and results are apt to be high With samples containing less than 10% of Pb, the Pb should be precipitated as $PbSO_4$ filtered redissolved and titrated as in the process of standardizing

(c) *Lead Carbonate and Lead Hydroxide* —Determine CO_2 by evolution with dilute HCl, absorbing in soda lime or KOH soln Calculate CO_2 to $PbCO_3$, subtract PbO equivalent from total PbO and calculate residual PbO to $Pb(OH)_2$

The following method of A N Finn (unpublished) gives total basicity of a pure white lead Place 2 g of pigment in an evolution flask, add a little CO_2 free H_2O, connect up to the separatory funnel and condenser (Knorr type), add thru the funnel, finally washing down, 100 cc of 0 25 *N* HNO_3 boil and absorb the CO_2 in soda lime tube in usual manner (having H_2SO_4 and $CaCl_2$ drying tubes in train) and weigh To the soln in the evolution flask add about 20 cc of neutral Na_2SO_4 soln and titrate with 0 25 *N* NaOH soln (carbonate free), using phenolphthalein CO_2 is calculated to $PbCO_3$ The amount of 0 25 *N* acid corresponding to the CO_2 is calculated and deducted from the total amount of 0 25 *N* acid neutralized by the sample and the difference is calculated to $Pb(OH)_2$

16 BASIC SULFATE OF LEAD[2]

Total Lead —Dissolve 1 g of the sample in 100 cc of 120 cc of 80% acetic acid, 95 cc of NH_4OH (sp gr 0 90) and 100 cc soln while hot, dilute with about 50 cc of H_2O and boil to 200 cc and titrate with standard NH_4 molybdate basic carbonate of Pb The NH_4 molybdate soln standardized against pure Pb foil, pure PbO

Total Zinc —Boil 1 g of the sample with 30 HCl (some $PbSO_4$ or $PbCl_2$ may not dissolve)

[1] This method of weighing lead sulfate

[2] J A Schaeffer's method *J Ind Eng Chem* 6 200

2 cc of a saturated soln of $Na_2S_2O_4$ and titrate with a standard soln of $K_4Fe(CN)_6$ in usual manner Calculate the Zn to ZnO [1]

99 70[2]—percentage of ZnO found = percentage of Pb constituents, then

$$\frac{\left(\frac{\text{Molecular weight } PbSO_4}{\text{Atomic weight Pb}} \times \text{Percentage of Pb found}\right) - \text{Percentage of Pb constituents}}{\text{Molecular weight } PbSO_4 - \text{Molecular weight PbO}} = \text{percentage of PbO present}$$

$$\frac{\left(\frac{\text{Molecular weight PbO}}{\text{Atomic weight Pb}} \times \text{Percentage of Pb found}\right) - \text{Percentage of Pb constituents}}{\text{Molecular weight PbO} - \text{Molecular weight } PbSO_4} = \text{percentage of } PbSO_4 \text{ present}$$

Molecular weight $PbSO_4$

ZINC OXIDE

17

Total Zinc—Dissolve 0 25 to 0 3 g in 10 cc of HCl and 20 cc of H_2O, make alkaline with NH_4OH then acid with HCl add 3 cc more of HCl dilute to about 250 cc with H_2O, heat nearly to boiling, and titrate with standard $K_4Fe(CN)_6$ soln as described by Low [3] Report as ZnO (includes Cd) Fe, Cu, or other interfering substances should be first removed as described by Low

Total Soluble Sulfur [4]—Moisten a 10 g sample with H_2O, add a few drops of Br and then HCl boil to expel Br, filter from any insoluble, and wash with hot H_2O Make alkaline with NH_4OH, then just slightly acid with HCl, heat to boiling, and add about 15 cc of hot $BaCl_2$ soln Let stand several hours (overnight), filter on a weighed Gooch crucible, wash well with hot H_2O, dry, ignite for 5 min cool, and weigh as $BaSO_4$ Calculate to S

LITHOPONE

18

(Ponolith Jersey Lily White Becton White Charlton White Orr s White)

Insoluble and Total Zinc—Take 1 g of the sample in a 200 cc beaker add 10 cc of HCl mix and add in small portions about 1 g of $KClO_3$, then heat on the steam bath until about half of the liquid is evaporated Dilute with H_2O, add 5 cc of H_2SO_4 (1+10) boil let settle, filter, wash ignite, cool, and weigh the insoluble which should be only $BaSO_4$ make a qualitative examination for Al_2O_3 and SiO_2 The in soluble should be examined under the microscope for the presence of natural crystalline barytes Sample may also be examined direct Make filtrate from insoluble alkaline with NH_4OH, acid with HCl add 3 cc of HCl, dilute to about 250 cc with H_2O, heat nearly to boiling and titrate with $K_4Fe(CN)_6$ soln as under zinc white Calculate to Zn

Zinc Oxide—Treat a 4 g sample of the lithopone for 4 hours with 200 cc of 1% acetic acid at room temp , stirring occasionally Filter by suction on a double filter paper and wash with cold H_2O, add to the clear filtrate 13 cc of NH_4OH, neutralize with HCl and then add 3 cc of HCl in excess Heat to boiling and titrate with $K_4Fe(CN)_6$ using uranium acetate soln as an outside indicator Calculate to ZnO

[1] It would probably be more accurate to remove total Pb as $PbSO_4$, and titrate the Zn in the filtrate after adding NH_4OH and HCl in usual manner

[2] 99 70 = average total percentage of $PbSO_4$ PbO and ZnO found in 70 total analyses of basic lead sulfate

[3] Low, Technical Methods of Ore Analysis

[4] Method of G Burr

Calculate this result to Zn subtract from total Zn, and calculate the difference to ZnS (Any $ZnCO_3$ or $ZnSO_4$ is included in the ZnO)

Zinc Sulfide [1]—Place 0 5 g of pigment in evolution flask with about 10 g of 'feathered'' or mossy Zn add 50 cc of H_2O, insert the stopper carrying a separatory funnel and an exit tube Run in 50 cc of HCl from the funnel having previously connected the exit tube to 2 absorption flasks in series first flask contains 100 cc of alkaline $Pb(NO_3)_2$ soln, second flask, 50 cc of same as a safety device After all the acid has run into the evolution flask heat slowly, finally boiling until the first appearance of steam in the first absorption flask, disconnect, let the PbS settle, filter wash with cold H_2O then with hot H_2O till neutral to litmus paper and washings give no test for Pb The PbS precipitate is dissolved in hot, dilute HNO_3 evaporated to fumes with H_2SO_4 and finally weighed as $PbSO_4$ Calculate PbS or $PbSO_4$ to ZnS

The alkaline Pb soln is made as follows Into 100 cc of KOH soln (50 g in 140 cc of H_2O) pour a saturated soln of $Pb(NO_3)_2$ (250 g in 500 cc of H_2O) until the precipitate ceases to redissolve stirring constantly while mixing About 3 volumes of the Pb soln will be required for 1 of the alkali

Instead of absorbing the evolved H_2S in alkaline $Pb(NO_3)_2$ soln a soln of 8 g of $CdCl_2$ in 250 cc of H_2O and 150 cc of NH_4OH (sp gr 0 90) may be used The CdS precipitate may be filtered on a weighed Gooch, washed with H_2O containing a little NH_4OH dried at 100 , and weighed Calculate to ZnS It is better to filter the CdS on a small filter and wash as above, then place filter and precipitate in a beaker and dissolve in HCl and $KClO_3$ (keeping at room temp at first), filter out any paper pulp or insoluble matter make filtrate alkaline with NH_4OH, then just acid with HCl heat to boiling and precipitate with $BaCl_2$ in usual manner Filter wash, ignite, and weigh $BaSO_4$ Calculate to ZnS

For very rapid work the contents of the absorption flask after all H_2S has been absorbed may be washed into a vessel with *cold* H_2O and diluted to about 1 liter, acidified with HCl and titrated with standard I soln Use starch indicator (The precipitate should be completely dissolved) The I soln is prepared by dissolving about 12 7 g of pure resublimed I and 18 g of KI in a little H_2O and then diluting to 1 liter

19

TITANIUM PIGMENT

Titanium oxide—Transfer 0 5 g of the dried sample to a 200 cc Pyrex beaker, add 20 cc of H_2SO_4 and 7–8 g of $(NH_4)_2SO_4$ Mix well and heat on hot plate until fumes of H_2SO_4 are evolved, and then continue the heating over a strong flame until soln is complete (usually requires not over 5 min of boiling) or it is apparent that the residue is composed of SiO_2, siliceous matter, or $BaSO_4$ Caution should be observed in visually examining this hot soln Cool the soln, dilute with 100 cc of H_2O, stir heat carefully to boiling while stirring let settle filter thru paper, and transfer the precipitate completely to the paper Wash the insoluble residue with cold 5% (by volume) H_2SO_4 until Ti is removed

Dilute the filtrate to 200 cc and add about 10 cc of NH_4OH (sp gr 0 90) to lower the acidity to approximately 5% H_2SO_4 (by volume)

Wash out a Jones reductor[2] with dilute 5% by volume H_2SO_4 and H_2O, leaving sufficient H_2O in the reductor to fill to the upper level of the Zn (These washings should require not more than 1 or 2 drops of 0 1 *N* $KMnO_4$ soln to obt in a pink color) Empty the receiver and put in at 25 cc (measured in a graduate) of ferric sulfate soln [Reagent 2,u)] Reduce the prepared Ti soln as follows [3]

[1] Evolution method of W G Scott White Paints and Painting Material p 207 see also Blair Chemical Analysis of Iron

[2] Directions for preparing a Jones reductor may be found in Blair The Chemical Analysis of Iron 6th Ed pp 88–90 or Treadwell Hall Analytical Chemistry Vol 2 5th Ed

[3] Lundell and Knowles The Determination of Titanium by reduction with Zinc and Titration with Permanganate *J Am Chem Soc* 45 2670 (1923)

(1) Run 50 cc of the 5% H_2SO_4 soln thru the reductor at a speed of about 100 cc per min

(2) Follow this with the T_1 soln

(3) Wash out with 100 cc of 5% H_2SO_4

(4) Finally run thru about 100 cc of H_2O Care should be taken that the reductor is always filled with soln or H_2O to the upper level of the Zn Gradually release the suction wash thoroly the glass tube that was immersed in the ferric sulfate soln, remove the receiver, and titrate immediately with 0 1 N $KMnO_4$ soln 1 cc of 0 1 N $KMnO_4$ = 0 0048 g of T_1 or 0 008 g of TiO_2 Run a blank determination using the same reagents washing the reductor as in the above determination Subtract this permanganate reading from the original reading and calculate the final reading to TiO_2 This will include Fe Cr, As and any other substance which is reduced by Zn and acid See calculations under 21 for reporting TiO_2

20

Iron oxide —Weigh a 1 g portion of the sample and treat as in 19 Transfer with out filtering to a graduated 200 cc flask, cool, fill to the mark with H_2O, mix let settle and determine Fe colorimetrically as follows Filter thru a dry filter paper discarding the first 20 cc, transfer 50 cc of the clear filtrate to a clean 100 cc Nessler tube or other comparator Add 1 drop or two of 0 1 N $KMnO_4$ soln to oxidize any ferrous Fe The faint pink color should persist for at least 5 min Add 10 cc of KCNS or $(NH_4)CNS$ soln dilute with H O to 100 cc and mix thoroly Compare the color immediately with a series of standards prepared side by side with the sample in similar tubes Prepare the standards from the standard ferric sulfate soln [Reagent 2(e)] so as to have a range of from 0 00000 g Fe to 0 00004 g Fe (0 5–4 0 cc Transfer the desired volumes of the standard ferric sulfate soln to 100 cc Nessler tubes containing 50 cc each of an acid soln (made up by dissolving 5 g of $(NH_4)_2SO_4$ in H_2O adding 20 cc of H SO_4, cooling diluting with H O to 200 cc and mixing) Add a drop of 0 1 N $KMnO_4$ soln (or sufficient to yield a pink color that will persist for 5 min), and then 10 cc of the thiocyanate soln Finally dilute all standards with H_2O to 100 cc and mix each thoroly

NOTE —For a single sample it is more convenient to run the standard Fe soln from a buret into a Nessler tube containing 50 cc of acid soln (made by dissolving 5 g of $(NH_4)_2SO_4$ in H_2O adding 20 cc of H_2SO_4, cooling and diluting with H O to 200 cc and mixing) a drop of 0 1 N $KMnO_4$ soln, 10 cc of the thiocyanate soln and then dilute with distilled H O until the depth of the color produced after diluting 100 cc and mixing exactly matches that of the sample I rom the buret reading calculate the amount of Fe When using standards the color comparisons must be made immediately

Calculate the total Fe found to Fe_2O_3 and report as such Calculate the TiO_2 equivalent by multiplying the Fe_2O_3 result by the factor 1 003 and subtract this figure from the total TiO_2 as determined in 19 and report the remainder as TiO_2

Report all results on the dry or moisture free basis

MIXED OR COMPOSITE PIGMENTS

MOISTURE (MATTER VOLATILE AT 105–110°)

21

Place 1–2 g of the sample in a wide mouthed short weighing tube provided with a glass stopper Heat with the stopper removed for 2 hours at a temp between 105 and 110 Insert the stopper cool and weigh Calculate the loss in weight as moisture (matter volatile at 105–110°)

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[1] On an extracted and dried portion of the determination is of little value If the original paint contained a part of the combined H_2O of the latter will be driven off in the drying of the extracted pigment and the moisture determination

22 LOSS ON IGNITION

Ignite 1 g of the pigment in a porcelain crucible over a Meker burner to constant weight [1]

23 INSOLUBLE MATTER

Moisten 1 g of the sample with a few drops of alcohol, cover, add 40 cc of HCl (1+1), boil gently 5–10 min Wash off cover, evaporate to dryness, and heat at about 150° for ½–1 hour to dehydrate the residue Moisten the residue with 4 cc of strong HCl, allow to stand a few min , dilute with 100 cc of hot H_2O, boil a few min , filter hot thru paper, wash with hot H_2O (till washings give no test for Pb and Cl) Ignite the paper and residue in a Pt or porcelain crucible, cool, and weigh total in soluble matter [2] (The insoluble matter may be filtered off on a Gooch crucible, washed with hot H_2O, dried at 105°, cooled, and weighed, then ignited, cooled, and weighed, when it is desired to get the loss on ignition (combined H_2O, organic matter, etc) of same, or the insoluble matter is not to be further examined) If the sample contains Ti pigment, practically all the TiO_2 will be found in the insoluble matter along with $BaSO_4$ and siliceous matter The TiO_2 may be determined in the insoluble matter or in a separate portion of the original sample by the method described in 19 and 20 To determine $BaSO_4$, mix the ignited insoluble matter with about 10 times its weight of anhydrous Na_2CO_3 (grinding the mixture in an agate mortar if necessary) and fuse the mixture in a covered Pt crucible, heating about 1 hour Let cool, place crucible and cover in a 250 cc beaker, add about 100 cc of H_2O, and heat until the melt is disintegrated Filter on paper (leaving crucible and cover in beaker) and wash the beaker and filter thoroly with hot H_2O to remove soluble sulfates Place the beaker containing the crucible and cover under the funnel, pierce the filter with a glass rod, and wash the residue into the beaker by means of a jet of hot H_2O Wash the paper with hot dilute HCl (1+1) and then with hot H_2O Remove crucible and cover, washing them with a jet of hot H_2O and removing any adhering precipitate Add cautiously 20 cc of concentrated H_2SO_4 and evaporate until fumes of H_2SO_4 are evolved and the precipitated matter is dissolved *Cool*, add cautiously, with stirring, about 100 cc of H_2O, and boil a few minutes Let the precipitate settle, filter on a weighed Gooch crucible, wash with hot H_2O, ignite, cool, and weigh as $BaSO_4$ Subtract the sum of the percentages of $BaSO_4$ and TiO_2 from the percentage of total insoluble matter and report the result as the percentage of insoluble siliceous matter [3]

If it is desired to examine the siliceous matter, unite the filtrates from the Na_2CO_3 fusion and the $BaSO_4$ precipitate, acidify with HCl evaporate to dryness, and proceed as in a silicate analysis taking cognizance of any TiO_2 that may be found, if Ti pigment were originally present

24 TOTAL LEAD ANTIMONY

Unite the filtrate and washings (total volume 150–200 cc) from the total insoluble matter, pass H_2S into the soln until it is saturated, add an equal volume of H_2O, and again saturate with H_2S Filter, wash with H_2O containing a little H_2S, dissolve in hot HNO_3 (1+3) washing the paper with hot H_2O, add 10–20 cc of H_2SO_4 (1+1), evaporate until copious fumes of H_2SO_4 are evolved, cool add about 75 cc H_2O, and then about 75 cc of 95% ethyl alcohol Stir let [illegible] for on a Gooch

[1] This determination may serve as a rough or appr [illegible] on
[2] See Ref 3 under 12
[3] Any soluble Al_2O_3 (Fe_2O_3) and in most cases MgO and [illegible] e me
pigment used MgO generally denoted the presence of an

wash with dilute alcohol and dry in an oven at 105–110 °, or, ignite gently in a radiator or muffle, cool, and weigh as $PbSO_4$. Calculate to PbO.[2]

If the pigment contains Sb filter and wash the sulfide precipitate as above, then wash the precipitate with a fine jet of H_2O from the paper into a porcelain dish or casserole, add 20 cc of NH_4 polysulfide [Reagent 2(j)], cover the vessel, and warm the mixture at 40–60° for 10–15 min with frequent stirring. Wash off cover, filter thru the paper used in the first case, and wash with 2–3% Na_2S or $(NH_4)_2S$ soln. Discard the filtrate. Dissolve the residue in hot dilute HNO_3 (1+3) and determine the lead as $PbSO_4$, as described above. Or, the original sulfide precipitate may be discarded and the Pb determined on a separate portion of the pigment as follows: To 1 g of the sample in a covered beaker, add 40 cc of HCl (1+1) and boil gently for 5–10 min. Wash off cover and evaporate to dryness. Moisten the residue with a few drops of HCl, add about 50 cc of hot H_2O, boil a few minutes, filter hot thru paper and wash with hot H_2O until washings give no test for Pb. (If the sample contains no insoluble matter, the filtration is omitted.) To the filtrate add 20 cc of H_2SO_4 (sp. gr. 1.84) and evaporate until dense white fumes of H_2SO_4 are copiously evolved. Allow to cool, but not below 60°, and then add slowly 50 cc of H_2O while the soln is agitated. Heat to boiling for several min in order to insure complete soln of Sb sulfate. Allow the $PbSO_4$ to settle out until the supernatant liquid is clear, not letting the temp. fall below 60°. If the liquid does not clear quickly it must be heated longer. When clear, pour the soln thru a weighed porcelain Gooch crucible with asbestos mat, decanting the soln as completely as possible without allowing more than a very small amount of $PbSO_4$ to go over into the crucible. Now add 10 cc more of H_2SO_4 (sp. gr. 1.84) to the $PbSO_4$ in the original beaker and boil for several min. Cool, add slowly 30 cc of H_2O, and again heat to boiling for a few minutes; allow the soln to cool to about 60° and completely transfer the $PbSO_4$ to the Gooch crucible with "lead acid" [Reagent 2(j)] to remove soluble sulfates and finally wash free of acid with dilute alcohol (equal parts of ethyl alcohol or denatured alcohol and H_2O). Dry in an oven at 105–110° or ignite gently in a radiator or muffle. Calculate to PbO, or determine as chromate as described below.

If soluble compounds of Ba or Ca are present $BaSO_4$ and $CaSO_4$ will be included with the $PbSO_4$. If soluble SiO_2 is present it will also be included with the $PbSO_4$ precipitate after washing with dilute alcohol may be dissolved in acid NH_4 acetate [Reagents 2(h)] and the Pb determined as $PbCrO_4$, as described below. For ordinary work the amount of $BaSO_4$ dissolved by the acetate treatment may be disregarded.

If the pigment contains no soluble Sb, Ba, or Ca compounds the Pb may be determined directly on the original pigment as follows: To 1 g of the sample in a covered beaker add 20 cc of HNO_3 (1+1), and boil gently a few min. Wash off cover, evaporate to dryness on a steam bath, moisten with HNO_3, add hot H_2O, and heat a few min. Filter, and wash with hot H_2O until washings are Pb free. Add 10–20 cc of H_2SO_4 (1+1) to the clear soln, evaporate, and determine Pb as $PbSO_4$, as above described.

In the absence of soluble compounds of Sb, Fe, Al, and Ba the following procedure may be used. Treat 1 g of the original pigment with 20 cc of HNO_3 (1+1) and proceed as above. To the clear soln, diluted to 200 cc, add NH_4OH in slight excess, acidify with acetic acid, and add 4–6 cc more of this acid; heat to boiling and add 10–15 cc of a 10% soln of $K_2Cr_2O_7$. Heat until the yellow precipitate assumes an orange color and let settle. Filter on a weighed Gooch crucible and wash by decanta-

[1] *U. S. Geological Survey Bulletin* 700, p. 33 (1919).

[2] It is not possible to determine the amount of basic lead carbonate and lead sulfate when carbonates or soluble sulfates of other metals, such as calcium, are present. Also neither basic lead carbonate nor basic lead sulfate is a definite compound.

taining the first filtrate Add a small piece of litmus paper acidify with HCl add 3 cc of HCl, heat nearly to boiling, and titrate with standard $K_4Fe(CN)_6$ as above

33 *Method II*

With pigments containing ZnO and ZnS, the ZnO may be determined as follows Weigh accurately 2 5 g of the pigment, transfer to a 250 cc graduated flask, moisten with a few drops of alcohol, add about 200 cc of 2–3% acetic acid, shake vigorously and let stand for 30 min at room temp , shaking once every 5 min Then let stand at room temp at least 5 hours, preferably overnight Fill to the mark with 2–3% acetic acid, mix, filter thru a dry paper, discard the first 25 cc and transfer 100 cc of the filtrate (corresponding to 1 g) to a 400 cc beaker To the clear soln add 30 cc of HCl (1+2), 100 cc of H O, and a small piece of litmus paper, add NH_4OH until slightly alkaline, render just acid with HCl then add 3 cc of HCl heat nearly to boiling and titrate with $K_4Fe(CN)_6$ as above Calculate the percentage of ZnO (any $ZnCO_3$ or $ZnSO_4$ is included in the ZnO) Subtract this result from the percentage of total Zn as ZnO, and calculate the difference to ZnS

34 SOLUBLE CALCIUM

Heat the united filtrate and washings, reduced in volume if need be, from the ZnS precipitate, to boiling and add 1 cc of NH_4OH and an excess of a hot saturated NH_4 oxalate soln Continue the boiling until the precipitate becomes granular, let stand about 1 hour filter, and wash with hot H O Ignite cool, and weigh as CaO,[1] [2] or place the beaker in which the precipitation was made under the funnel, pierce the apex of the filter with a stirring rod, and wash the precipitate into the beaker with hot H_2O, pouring warm H SO_4 (1+4) thru the paper and washing a few times Add about 30 cc of H_2SO_4 (1+4) dilute to about 250 cc heat to 90°, and titrate at once with standard (0 1 N) $KMnO_4$ soln (the temp of the soln should not be below 60 when the end point is reached See Reagents) Calculate to CaO [3] (The Fe value of $KMnO_4 \times 0.502 = CaO$ value)

35 SOLUBLE MAGNESIUM

Acidify the filtrate from the Ca precipitate with HCl, add 10 cc of a saturated soln of $Na(NH_4)HPO_4$ and NH_4OH dropwise, with constant stirring When the crystalline $(NH_4)MgPO_4$ has formed, add 5 cc excess of NH_4OH Allow the soln to stand in a cool place for not less than 4 hours, preferably overnight [4] filter and wash with H_2O containing 2 5% of NH_3 Dissolve the precipitate in a small quantity of hot dilute HCl dilute the soln to about 100 cc with H O, add 1 cc of a saturated soln of $Na(NH_4)HPO_4$ and NH_4OH dropwise, with constant stirring, until the precipitate is again formed as described, and then add 5 cc excess of NH_4OH Let the precipitate stand in a cool place for not less than 2 hours, filter on a Gooch crucible, wash with H_2O containing 2 5% of NH_3, ignite cool and weigh as $Mg_2P_2O_7$ [5] Calculate to MgO

[1] Care must be exercised in this washing as 1000 cc of boiling water will dissolve over 0 01 g of CaC O
[2] For more accurate work the CaC O precipitate should be ignited cooled cautiously moistened with H O redissolved in HCl and the soln diluted to 100 cc Then NH OH should be added in slight excess the liquid boiled and filtered and washed if a precipitate appears Then reprecipitate the Ca with NH OH and (NH) C O as above filter wash ignite cool and weigh or titrate as described See also Ref 3 under 1
[3] See Ref 3 under 12 also Refs 2 and 3 under 26
[4] The less the amount of Mg present the longer the precipitate must be allowed to settle
[5] If the sample contained Mn it will be caught in large part with the Mg NH O If desired Mn may be determined by dissolving the $Mg_2P_2O_7$ in HNO_3 and applying the bismuthate method

36 CARBON DIOXIDE

Use from 1 to 2 g of the pigment depending upon the probable CO content,[1] following either of the methods described under the Determination of Carbon Dioxide in the Standard Methods of Chemical Analysis of Limestone, Quicklime and Hydrated Lime (A S T M Designation C 25) of the American Society for Testing Materials.[2]

37 TOTAL SOLUBLE SULFUR COMPOUNDS[3]

Treat 1 g of the pigment in a 400 cc beaker with 10 cc of H_2O, 10 cc of HCl saturated with Br, and 5 g of NH_4Cl, digest (covered) on a steam bath for 5 min, dilute with hot H_2O to about 200 cc, boil for 5 min filter to separate any insoluble matter and wash thoroly with hot H_2O Nearly neutralize the clear soln in a covered beaker with NaOH soln, complete the neutralization with dry Na_2CO_3, and add about 2 g more of this reagent Boil 10–15 min, wash off cover, let settle, filter, and wash with hot H O Re dissolve the precipitate in HCl (1+1), reprecipitate with Na_2CO_3 as above, filter, and wash thoroly with hot H_2O Acidify the united filtrates with HCl, adding about 1 cc in excess Boil to expel Br and to the clear boiling soln add slowly with stirring an excess of a 10% $BaCl_2$ soln Let stand on a steam bath for at least 1 hour, filter on a weighed Gooch crucible wash thoroly with boiling H_2O, dry, ignite at a dull red heat, cool and weigh as $BaSO_4$ This will include soluble sulfates SO_3 formed from SO_2 and the SO_3 that is formed from sulfide S.[4]

38 SOLUBLE SULFATE[5]

Treat 1 g of the pigment with 10 cc of H_2O, 10 cc of HCl, and 5 g of NH_4Cl Boil until H_2S is expelled adding more HCl (1+1) if necessary, dilute with hot H_2O to about 200 cc boil for 5 min, filter to separate any insoluble matter and wash thoroly with hot H_2O Nearly neutralize the clear soln with NaOH soln and make a double precipitation with Na_2CO_3, as in preceding method finally weighing as $BaSO_4$ as described above.[6]

39 SULFIDE SULFUR[5]

Place 0.5–1 g of the pigment in a flask with about 10 g of feathered or mossy Zn, and add 50 cc of H_2O insert a stopper carrying a separatory funnel and an exit tube Run in 50 cc of HCl from the funnel, having previously connected the exit tube to 2 absorption flasks in series, the first flask contains 100 cc of alkaline $Pb(NO_3)_2$ soln [Reagent 2(n)], the second flask 50 cc of the same soln as a safety device After all the acid has run into the evolution flask heat slowly finally boiling, until the first appearance of steam in the first absorption flask Disconnect, let the PbS settle filter and wash with cold H_2O then with hot H_2O till neutral to litmus paper and washings give no test for Pb Dissolve the PbS precipitate in hot dilute HNO_3 and determine the Pb as $PbSO_4$ Calculate to S For very rapid work, the evolved H_2S may be absorbed in an ammoniacal $CdCl_2$ or $ZnSO_4$ soln [Reagent 2(o)] contained in 2 flasks connected in series the contents of the absorption flasks washed into a vessel with cold H_2O and diluted to about 1 liter, acidified with HCl, and ti

If the sample is high in sulfide e g contains a high percentage of lithopone grind 1–2 g of the pigment with dry As_2O_3 transfer to the evolution flask add 50 cc of H O and run in H SO (1+1) from the separatory funnel Or place at the front of the purifying and drying train a tube containing acidified CuSO soln in MnO soln or CrO_3 soln

[1] 1929 Supplement to Book of A S T M Standards

[2] See Ref 3 under 12

[3] See Ref 1 under 21

[4] See 1 at 3 under 12

[5] Volumetric Method of W O Scott White Paints and Painting Materials p 37" see also Blair The Chemical Analysis of Iron The percentage of sulfide sulfur can be calculated from the percentages of total S in and Zn soluble in 2–3 % acetic acid assuming the sulfide to be ZnS See 33

trated with standard KIO_3 soln [Reagent 2(p)], using starch indicator [Reagent 2(q)]

40 SULFUR DIOXIDE[1]

Transfer 10 g of the pigment to a suitable flask, insert a stopper fitted with a separatory funnel and a spray trap delivery tube,[2] and attach the latter to a condenser Place about 150 cc of HCl (1+3) in the funnel, the stopcock being closed,[3] and connect the other end of the condenser with a delivery tube which passes thru a 2 hole stopper and extends nearly to the bottom of an absorption flask, thru the other hole of the stopper connect a tube or flask to serve as a safety device Place 25 cc of 0 05 *N* I soln [Reagent 2(s)] in the absorption flask (dilute with H_2O if need be) and 20 cc of 10% KI soln in the safety tube, fit stopper in the absorption flask Open the stopcock and allow the acid to slowly enter the flask Before all the acid is admitted, air (washed with NaOH soln) is forced thru the top of the separatory funnel (about 2 bubbles per second in the KI soln) Boil the soln 3 min with the air passing thru, then remove the source of heat and pass air thru for 30 min Disconnect the absorption vessels, wash the KI soln into the I soln, and titrate at once with 0 05 *N* $Na_2S_2O_3$ soln, using starch indicator Run a blank determination in exactly the same manner except for the omission of the pigment Subtract this figure from the previous one and calculate the final result to SO (1 cc of 0 05 *N* I = 0 0016 g of SO_2)

41 MATTER SOLUBLE IN WATER

Transfer 2 5 g of the pigment to a graduated 250 cc flask, add 100 cc of H_2O and boil for 5 min Cool to room temp , dilute to the mark with H_2O, mix, and allow to settle Filter the supernatant liquid thru a dry filter paper and discard the first 20 cc of the filtrate Transfer 100 cc of the clear filtrate to a weighed dish, evaporate to dryness on a steam bath, dry for 1 hour in an oven at 105–110°, cool, and weigh Calculate the percentage of water soluble matter (The nature of this may be determined by further examination, as the percentages of SO_3 and CaO may be indicative)

42 CALCULATIONS

The calculation of the component pigments of a mixed or combination pigment may be difficult Depending upon the complexity of the mixed pigment, certain assumptions must be made as to the composition or formulas of component pigments and as to the manner in which the acidic and basic radicles are combined Add any Al_2O_3 (Fe_2O_3) found in the soluble portion to the siliceous matter and report the sum as "Insoluble siliceous matter ' unless the soluble Al is high, in this case, an aluminate is probably present, and the Al_2O_3 should be reported as Al_2O_3 If a small amount of soluble Mg is found it should also be added to the siliceous matter If the soluble Mg is high, the presence of $MgCO_3$ is indicated, and the MgO is calculated to $MgCO_3$ as pointed out below The insoluble siliceous matter reported should be based on the weight obtained on drying the total insoluble matter at 105° if the combined H_2O contained therein is to be considered

In the absence of ZnS or TiO report $BaSO_4$ as $BaSO_4$ If ZnS is present, calculate the $BaSO_4$ equivalent by multiplying by 2 85, report sum of ZnS + $BaSO_4$ as "litho

[1] This method is not applicable in the presence of sulfides decomposable under the conditions given

[2] A Knorr CO_2 apparatus is convenient In this case the vertical condenser may be connected with an absorption tower containing the I soln followed by the KI soln in a suitable tube

[3] To minimize if not eliminate any possible oxidation by the air add about 1 g (in one piece) of $NaHCO_3$ to the evolution flask then add the acid directly to the flask omitting the separatory funnel and the current of air Boil the soln until about 50 cc of distillate has passed over

pone ' If TiO_2 is present, calculate the $BaSO_4$ equivalent by multiplying by 3 17, report sum of TiO_2+$BaSO_4$ as "titanium pigment" Report residual $BaSO_4$ as $BaSO_4$ If TiO_2 is present and $BaSO_4$ is absent or is present in a smaller amount than would be indicated by the above factor, then report TiO_2 as TiO and $BaSO_4$ as $BaSO_4$ If $CaCO_3$, $CaSO_4$, $BaCO_3$, and $MgCO_3$ are absent, calculate CO_2 to basic carbonate white lead, $(PbCO_3)_2$ $Pb(OH)_2$, and soluble SO_3 to $PbSO_4$ Any excess of Pb is calculated to PbO, added to the $PbSO_4$ and the sum is reported as basic $PbSO_4$, or multiply the sum of $PbSO_4$+PbO by 0 08S to obtain the ZnO, add this result to the $PbSO_4$+PbO and report as basic sulfate white lead (The ZnO factor is based on the assumption that the average composition of commercial basic sulfate white lead is 78 5% $PbSO_4$, 16 0% PbO, and 5 5% ZnO) Lead oxide (PbO) should not be reported except in the presence of $PbSO_4$ unless the entire analysis is reported in the elementary or oxide form

If the sample contains CO_2 but no soluble SO_3 calculate total Pb to basic carbonate white lead $(PbCO_3)_2$ $Pb(OH)_2$, calculate residual CO_2 to $CaCO_3$, then to $BaCO_3$ and $MgCO_3$ if soluble Ba and Mg should be present in sufficient amounts to indicate the presence of these carbonates The CO_2 result will be an index of this A small amount of residual CaO is probably from the siliceous matter and should be added to the insoluble siliceous matter

A small amount of soluble Ba may be from the $CaCO_3$ used or may be due to the solubility of $BaSO_4$ if this compound is present in the original pigment This Ba may be calculated to $BaSO_4$ and added to the $BaSO_4$ found in the insoluble matter

If the sample contains soluble SO_3 but no CO_2 calculate CaO to $CaSO_4$ or $CaSO_4$ $2H_2O$, residual SO_3 to $PbSO_4$, add residual PbO to $PbSO_4$ and report sum as basic $PbSO_4$, or multiply $PbSO_4$+PbO by 0 058 and add the result to the $PbSO_4$+PbO, and report the total as basic sulfate white lead

If the sample contains $CaCO_3$ ($MgCO_3$ $BaCO_3$) and also basic sulfate white lead, or $CaSO_4$ and basic carbonate white lead or a mixture of these it is not possible to determine or calculate the amount of $PbCO_3$ or $PbSO_4$ with any degree of certainty [1] The presence of appreciable amounts of CaO and SO_3 in the water soluble matter indicates the probable presence of $CaSO_4$ in the original pigment The following arbitrary calculations may be made calculate water soluble SO_3 to $CaSO_4$ or $CaSO_4$ $2H_2O$ subtract this SO_3 from total soluble SO_3 and calculate the remainder to $PbSO_4$, calculate residual CaO to $CaCO_3$ and then residual CO_2 to $(PbCO_3)_2$ $Pb(OH)_2$ If there is an excess of CO_2, calculate to $MgCO_3$ or $BaCO_3$ if the amounts of soluble Mg and Ba indicate the probable presence of these carbonates Add residual PbO to $PbSO_4$ and calculate as above, to basic sulfate white lead The procedure followed by the Federal Specifications Board should be noted [2]

Report total Sb as Sb_2O_3

Calculate sulfide S to ZnS, subtract the Zn equivalent to the S from the total Zn then subtract the Zn required for the basic sulfate white lead, and report the remainder as ZnO

Report moisture loss on ignition SO_3 and matter soluble in H_2O directly

[1] See Ref 7 under 16 and Ref 3 under 12

[2] Federal Specifications Board Specification No 106 for White Paint and Tinted Paints Made on a Semipaste and Ready Mixed U S Bur Standards Circ C89 2d ed p 2 The total lead in a sample is dissolved by dilute acetic acid and hot ammonium acetate (It is not possible to determine the amount of white lead) The total lead and this weight multiplied by the factor 0 853 shall be considered white lead (It is not possible to determine the amount of PbCO nor basic PbSO) The factor to convert PbSO to (PbCO)2 Pb(OH)2 is 0 854 and to convert PbO to (PbCO)2 Pb(OH)2 is 0 913 The arbitrary factor used under this specification is the mean of the largest and smallest of these 3 factors)

X LEATHERS—TENTATIVE

(These methods are essentially the same as the official and provisional methods of the American Leather Chemists Association)

VEGETABLE TANNED LEATHER

1 PREPARATION OF SAMPLE[1]

Reduce the leather by slicing, planing, cutting, shredding, or rasping to as fine a state of subdivision as is practicable Avoid heating the sample during its preparation Do not use the ordinary type of grinding mill Spread out the prepared sample and allow it to return to atmospheric moisture condition, mix thoroly, and place in tightly covered containers

MOISTURE[2]

2 *Method I*

Place 5-10 g of the sample, as prepared under 1, in a tared, wide, shallow weighing bottle (or a similar dish, which can be covered tightly), and dry in an electric oven for 15 hours at 100–102° Cover the weighing bottle, cool in a desiccator containing concentrated H_2SO_4 and weigh The moisture in the leather as received may be determined by quickly cutting a representative portion of the sample into small pieces and drying as directed without further preparation

Method II—By Toluene Distillation

3 APPARATUS

(a) 500 cc flask —Erlenmeyer or distilling flask of Pyrex or other resistant glass

(b) *Receiving tube* —Graduated in tenths of a cc

(c) *Liebig condenser* —Sealed in, straight tube, about 25 cm (10 inches) long, with delivery tube approximately 9 5 mm (0 375 inch) in diameter

Assemble the apparatus as shown in XXVII, 3 Before each distillation clean the condenser and receiving tube with CrO_3-H_2SO_4 mixture, rinse thoroly with H_2O, then with alcohol, and dry in an oven or with a current of air Calibrate the receiving tube by distilling toluene containing known quantities of H_2O Read the volume of H_2O to 0 01 cc

4 DETERMINATION

Weigh 20 g of the prepared sample and transfer to the distilling flask Immediately add about 200 cc of dry toluene having a boiling point, under normal pressure, of 110–112°, and connect the flask with the receiving tube and condenser Fill the receiving tube with toluene pouring it thru the condenser Heat the distilling flask gently and distil at the rate of about 4 drops per second for exactly 2 hours At the end of 1, 1 25, 1 5, 1 75, and 2 hours' distillation, wash down the condenser by pouring toluene in at the top while brushing thoroly with a tight haired, close fitting tube brush that has been boiled previously in toluene (A long handle may be made by fastening to the brush a piece of heavy Cu wire) At the end of 2 hours disconnect the receiving tube, dislodge any drops of H_2O on the inside by rubbing with a piece of light Cu wire twisted at one end into a loop, and allow the tube to come to room temp Read the volume of H_2O to 0 01 cc and make such calibration correction as may be necessary Assuming that 1 cc of H_2O weighs 1 g, calculate the percentage of moisture

5 TOTAL ASH[1]

Incinerate slowly 5 g of the sample, as prepared under 1, at a dull red heat If difficulty is experienced in burning off the C, leach the residue with hot H_2O, filter on an ashless filter, dry and ignite the filter and residue, add the filtrate, evaporate to dryness, and ignite Cool in a desiccator containing concentrated H_2SO_4 and weigh

The ash may be examined for acids and bases by any suitable method Al, Mg[4] Na, Ba, Ca, and Pb are the bases, and HCl and H_2SO_4 are the acids which it may be necessary to determine

6 INSOLUBLE ASH[1]

Air dry the leather remaining after the extraction of water-soluble material as directed under 9 and weigh Incinerate slowly a portion equal to exactly ¼ of the total weight until all C is burned off, cool in a desiccator containing concentrated H_2SO_4, and weigh Calculate the insoluble ash on the basis of the original leather represented

7 PETROLEUM ETHER EXTRACT[1]

Place 5 g of the leather, as prepared under 1, in a fat free paper thimble, cover with a layer of fat free cotton, and extract in a Johnson or Soxhlet extractor for 8–10 hours with petroleum ether, distilling between 50 and 80° Heavily greased leathers (containing 15% or more fat) will require the maximum time Remove the receiving flask, evaporate the petroleum ether on a steam bath, and dry the residue at 98–100° for periods of ½ hour each until a practically constant weight is obtained Avoid prolonged continuous heating resulting possibly in the partial volatilization or oxidation of the extract

8 MINERAL ACIDITY[1]

(Modified Procter and Searle method[7])

Weigh 2 g of the leather as prepared under 1, into a Pt dish add 40 cc of a 0 1 *N* Na_2CO_3 soln mix thoroly, and evaporate to complete dryness on a steam bath Incinerate the residue at a dull red heat preferably in a muffle furnace, until the C has been nearly burned off let cool, moisten carefully with hot H_2O, adding about 25 cc, and break up lumps with a glass rod Filter into a 300 cc flask thru an ashless paper and wash 4 or 5 times with hot H_2O Return the filter paper and residue to its dish, dry, and ignite until all C is burned off Cool and add to the residue from a buret a quantity of 0 1 *N* H_2SO_4 exactly equivalent to the Na_2CO_3 originally added Cover the dish and place on a steam bath for 30 min Filter, if necessary, into the flask containing the first filtrate washing the paper thoroly with hot H_2O until free from acid Cool the soln and add 2 or 3 drops of methyl orange indicator If the soln is alkaline no further titration is necessary and the acidity is stated as "none" If the soln is acid titrate to a distinct yellow color with the 0 1 *N* Na_2CO_3 soln Express the result as percentage of H_2SO_4 With each set of determinations run a blank thru the entire procedure using the standard solns If the blank is over 0 3 cc, repeat the determinations

9 EXTRACTION OF WATER SOLUBLE MATERIAL[1]

Weigh 30 g of the leather prepared as directed under 1 (If the fat content of the sample, as determined by the petroleum ether extract, is more than 6%, extract the 30 g charge with petroleum ether distilling between 50 and 80°, and allow the petroleum ether to evaporate spontaneously from the charge before proceeding with the extraction of water soluble material) To the charge add sufficient H_2O to soak thoroly and cover it and mix well Transfer the leather and extract to a percolator

that may be kept at 50° (The Reed Churchill extractor[9] is especially convenient) Extract at 50° by percolating with H_2O at 50°, collecting 2 liters of percolate in 3 hours Cool to room temp, dilute to exactly 2 liters, and mix thoroly

To the extract, prepared as above, add a few drops of toluene to prevent fermentation and reserve for the determination of glucose, soluble solids, and soluble non tannins

GLUCOSE[10]

10 REAGENTS

(a) *Di potassium phosphate* —Use only K_2HPO_4 that is practically free from primary and tertiary salts, has been dried in thin layers at 98–100° for 16 hours, and kept in tightly stoppered bottles A soln of the salt should have a pH value of about 9 0 and give a barely perceptible pink with phenolphthalein indicator

(b) *Neutral lead acetate soln* —Prepare as directed under XXXIV, 18 (d)

(c) *Soxhlet's modification of Fehlings soln* —Prepare as directed under XXXIV, 31

(d) *Phenolphthalein soln* —Dissolve 0 5 g of phenolphthalein in 100 cc of 95% alcohol

(e) *Tartaric acid* —Grind pure tartaric acid to a fine powder

11 PREPARATION OF SOLUTION

To 200 cc of the leather extract, prepared as directed under 9, add by means of a pipet 25 cc of the neutral Pb acetate soln Shake frequently for 5–10 min, then filter at once thru a dry, folded filter, returning the filtrate until it is clear Keep the containers and funnel covered during these operations Add to the filtrate 5 5 g of the dried K_2HPO_4 (The quantity of K_2HPO_4 must be not less than 4 5 nor more than 6 5 g) Shake frequently for 3–5 min, until all the phosphate has dissolved, then filter thru a dry, folded filter, returning the first runnings until the filtrate clears, and letting the funnel drain well Pipet 150 cc of the filtrate into a 500 or 600 cc Erlenmeyer flask, and add by means of a pipet 7 5 cc of HCl (sp gr 1 18) Also add about 25 mg of powdered stearic acid or 5–10 drops of kerosene to control frothing and boil under a reflux condenser for exactly 2 hours (Should the soln foam when it begins to boil turn off the flame at the first indication of foaming and when foaming subsides relight immediately No further trouble should be experienced After hydrolysis the acid soln may be allowed to stand at laboratory temp overnight without risk of loss of sugar) Cool to 10–15°, add 2 drops of phenolphthalein indicator, carefully neutralize with NaOH (1+1) added from a buret, and then add 0 5 cc in excess *Without delay* transfer to a 200 cc volumetric flask, complete to volume with H_2O, and filter thru a double filter, returning the filtrate until it is clear *During filtration keep the filtrate just acid by the addition from time to time of small quantities of pulverized pure tartaric acid Immediately* determine the dextrose in the soln

12 DETERMINATION

Pipet 50 cc of the soln, as prepared under 11, into a mixture of 25 cc of the Cu soln and 25 cc of the alkaline tartrate soln and proceed as directed under XXXIV, 38 Express the results as percentage of glucose on the leather basis, the 50 cc aliquot being equivalent to 0 5 g of leather

13 SOLUBLE SOLIDS

If the H O extract, as prepared under 9, is clear, proceed as directed under XI, 2, if cloudy proceed as directed under XI, 4

14 SOLUBLE NONTANNINS

Determine as directed under XI, 7

15 SOLUBLE TANNIN

The percentage of soluble tannin is the difference between 13 and 14

16 NITROGEN

Determine as directed under II, 20

17 HIDE SUBSTANCE

Multiply the percentage of N as obtained under 16, by the factor 5 62, to convert to the percentage of hide substance

18 COMBINED TANNIN

Deduct the sum of the percentages of moisture, under 2 or 3, insoluble ash, under 6, petroleum ether extract under 7, soluble solids, under 13, and hide substance, under 17, from 100 The remainder is the percentage of combined tannin

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[2] Ibid 16, 547 (1921) 17, 262 (1922) 19, 568 (1924), 20, 334 (1925) 21, 435 (1926), 22, 265 (1927) J Assoc Official Agr Chem , 10, 31, 143 (1927)
[3] J Am Leather Chem Assoc 13, 7 (1918), 14, 243 (1919) 15, 130, 270 (1920)
[4] Ibid , 16, 595 (1921) 17, 274 592 (1922)
[5] Ibid 14, 140 499, 507 (1919), 16, 458 (1921) 17, 292, 540 (1922), J Soc Leather Trades Chem , 4, 300 (1920)
[6] Procter, Leather Industries Laboratory Book 2nd ed , 1908 p 369, J Am Leather Chem Assoc , 14, 330 (1919)
[7] J Am Leather Chem Assoc 17, 88 (1922) 18, 430 (1923)
[8] Ibid 11, 219 (1916) 13, 142 (1918), 14, 488 (1919), 15, 581 (1920), 16, 124, 264 491 (1921) 17, 220 (1922)
[9] Ibid 14, 133 (1919)
[10] Ibid , 7, 645 (1912) 9, 421 (1914) 15, 411 (1920), 16, 480 (1921), 17, 284 1922) 18, 262 459 (1923) 19, 237 339 (1924)

XI TANNING MATERIALS[1]—TENTATIVE

(These methods are essentially the same as the official and provisional methods of the American Leather Chemists Association)

EXTRACTS

1 PREPARATION OF SOLUTION

(a) *Solid and powdered extracts*.—Grind the sample, if necessary, as rapidly as possible in a porcelain mortar until all will pass a 10 mesh sieve of Cu or brass, mix thoroly, and bottle. Weigh rapidly a quantity of sample containing 4 g of tannin (not less than 3.75 g nor more than 4.25 g).[2] Transfer to 100 cc of H_2O at 85°, place on a steam bath, cover, and heat. Stir frequently until a homogeneous soln or suspension is obtained. Wash into a 1 liter flask with 800 cc of H_2O at 85°. Allow to cool overnight at a temp. not below 19°, bring to 20° by placing the flask in H_2O, the temp. of which is not below 19°, and dilute to 1 liter.

(b) *Liquid extracts*.—Let the sample come to room temp., mix thoroly, and weigh rapidly a charge yielding the same quantity of tannin as specified under (a). Dissolve by washing into a liter flask with 900 cc of H_2O at 85°. Allow to cool and dilute to 1 liter at 20°, as described under (a).

After the preparation of the soln. proceed at once with the analysis.

2 TOTAL SOLIDS

Thoroly mix the soln. as prepared under **1**, pipet at once 100 cc into a weighed flat bottomed glass dish, 2¼–3 inches in diameter, and (1) evaporate and dry for 16 hours in a combined evaporator and dryer[3] at 98–100°, or (2) evaporate on a steam bath and then dry for 12 hours on the bottom of a water oven at 98–100°. Remove immediately to a desiccator containing concentrated H_2SO_4 (place no more than 2 dishes in one desiccator) and weigh rapidly when cooled. Calculate the percentage of total solids.

SOLUBLE SOLIDS

3 PREPARATION OF FILTER

To about 75 cc of the soln., as prepared under **1**, add 1 g of kaolin. (The kaolin used should be neutral to phenolphthalein and should not yield more than 1 mg of soluble solids per 100 cc of filtrate of a 1% suspension in H_2O after an hour's digestion at 20°.) Stir, and pour immediately into a single, 15 cm. No. 590 S. & S. or No. 1F Swedish folded filter.[4] (These papers must be pleated by hand as they are not available in folded form.) Return the filtrate to the paper when approximately 25 cc has run thru and repeat the operation for an hour, thus transferring all the kaolin to the paper. At the end of an hour discard the soln. on the filter by siphoning it off, disturbing the kaolin as little as possible. An ordinary wash bottle serves well for this purpose.

4 DETERMINATION

Bring about 150 cc of the original soln., as prepared under **1**, to exactly 20°. Fill the filter, prepared as directed under **3**, with this soln. and discard the filtrate until it runs thru clear. Keep the filter full. the temp. of the filtering soln. at 20–25°, and the funnel and receiving vessel covered. Pipet at once 100 cc of the clear filtrate into a weighed dish, evaporate, and dry as directed under **2**. Calculate the percentage of soluble solids.

INSOLUBLE SOLIDS

5 The percentage of insoluble solids is the difference between 2 and 4

NONTANNINS

REAGENT

6 *Hide powder*[8].—This should be of woolly texture, well delimed, and 10 g of the water free powder should require 12–13 cc of 0 1 *N* NaOH to neutralize it Calculate the quantity of air dried hide powder that will be required for the number of determinations to be made, on the basis of 12 5 g of H_2O free powder for each determination Increase this calculated amount by 10 g of air dried hide powder to provide a sufficient quantity for the determination of moisture in the wet chromed hide powder and also for a working leeway)

Digest the total quantity of air-dried hide powder with 10 times its weight of H_2O until thoroly soaked Then, for each g of the air dried hide powder so digested, add 1 cc of a 3% chrome alum soln ($K_2SO_4Cr_2(SO_4)_3 \cdot 24H_2O$) and either agitate frequently for several hours and let stand overnight, or agitate in some form of mechanical shaker for an hour[8] Transfer to a strong linen filter and squeeze thoroly, using the linen filter as a bag leave the hide powder in it and digest for 15 min with a quantity of water equal to 15 times the weight of the air dried hide powder used Filter, and squeeze to approximately 73% of H_2O, using a press if necessary Very strong pressure is required to reduce the water content below 70% Repeat the digestion and filtration 3 times The wet chromed hide powder, as finally prepared, shall contain as nearly as possible 73% of H_2O The moisture content must be not less than 72% nor more than 74%[8] Determine moisture in 20 g of the squeezed hide powder as directed under 2

DETERMINATION

7 Place 46 g of the wet hide powder prepared as directed under 6, in a shaker bottle of suitable capacity, add 200 cc of the tanning soln, prepared as directed under 1, and shake immediately for 10 min in a mechanical shaker Squeeze at once thru linen add 2 g of kaolin as used under 3, to the filtrate that contains the nontannins stir and filter thru a single folded 18 5 cm filter paper (No 1F Swedish preferred), refiltering until the filtrate is clear Test the filtrate with gelatin salt soln (1% gelatin and 10% salt) and if a precipitate forms, report the fact Pipet 100 cc of the filtrate into a weighed dish and evaporate as directed under 2 Correct the weight of the nontannin residue for the dilution caused by the H O retained in the wet hide powder Calculate the percentage of nontannins

TANNIN

8 The percentage of tannin is the difference between 4 and 7

SUGARS[7]

REAGENTS

9 The reagents and solns are described under X, 10

PREPARATION OF SOLUTIONS

10 To 100 cc of soln as prepared under 1, add 50 cc of saturated neutral Pb-acetate soln shake well and let stand for from 5 to 10 min Filter thru a folded filter (18 5 cm), returning the filtrate until it is clear Let the filter drain for about 30 min after all the soln has been poured Remove the excess Pb from the filtrate with dried K_2HPO_4 using the phosphate in the proportion of 5 g to 200 cc of the filtrate (Meas-

ure the filtrate in a graduated cylinder Usually from 360 to 380 cc will be obtained, requiring from 9 to 9 5 g of K_2HPO_4 Weigh the phosphate to within 0 1 g) After adding the phosphate, shake well for 4 or 5 min and filter thru a folded filter (18 5 cm)

11 DETERMINATION

Reducing sugars —Place 100 cc of the clarified deleaded soln obtained under 10 in a flask, add 33 3 cc of H_2O and if the reduction is not made at once, 8–10 drops of toluene, shake well and stopper with a plug of cotton Keep in a cool place and make the reduction within the following 24 hours When ready for reduction, filter if toluene has been added Determine reducing sugars in duplicate 50 cc aliquots, as directed under XXXIV, 38 After correcting the weight of the Cu_2O for the blank of the Fehlings soln, find the equivalent milligrams of dextrose from XLII, 9 To express as percentage of dextrose, multiply the milligrams of dextrose by 3 and divide the result by grams of sample per liter of soln

12

Total sugars —Place 150 cc of the clarified, deleaded soln obtained under 10 in a 500 cc Erlenmeyer flask, add 7 5 cc of HCl (sp gr 1 18), and boil under a reflux condenser for exactly 1 hour (If the soln foams at the start, which is unusual, add 5–10 drops of kerosene) After boiling for an hour, remove the flask, stopper loosely when moderately cool, and let stand until ready for reduction, usually over night Cool the soln in ice H_2O for from 20 to 30 min , add 2 drops of phenolphthalein soln, carefully neutralize with NaOH (1+1) and then add HCl dropwise until the color of the indicator is just discharged After bringing the soln to room temp , transfer it to a 200 cc flask make to mark, mix, and filter until clear Reduce Fehling's soln with duplicate 50 cc aliquots and calculate results as directed under 11

13

Non reducing sugars —The percentage of non reducing sugars is the difference between 11 and 12

DETECTION OF SULFITE-CELLULOSE[8]

14 REAGENTS

Sulfite-cellulose soln —Dissolve 0 5 g of the total solids derived from sulfite cellulose in 1 liter of H_2O and add sufficient tanning material, free from sulfite cellulose, to give a concentration of 3 75–4 25 g of tannin per liter

15 DETERMINATION

Place 5 cc of the tanning soln, prepared as directed under 1, in a test tube Add 0 5 cc of aniline and shake well, then add 2 cc of HCl and mix again Compare the precipitate formed with that produced when the sulfite cellulose soln is similarly treated In the predetermined absence of the synthetic tanning material, Neradol D, sulfite cellulose is held to be present if the volume of the precipitate approximately equals or exceeds that of the comparison soln

LIQUORS

16 PREPARATION OF SOLUTION

Dilute the liquor with H_2O at room temp to contain approximately 0 7 g of solids in 100 cc of soln If the liquor does not give a proper soln with H_2O at room temp , it may be diluted with water at 80° and then cooled to 20°, as directed under I (a)

TOTAL SOLIDS

17 Proceed as directed under **2**

SOLUBLE SOLIDS

18 Proceed as directed under **4**

NONTANNINS

19 Proceed as directed under **7**, using the quantity of wet chromed hide powder that will give the ratio between the tannin and hide powder shown in the following table:[9]

TANNIN RANGE PER 100 cc	DRY HIDE POWDER PER 200 cc
gram	gram
0 35–0 45	9 0–11 0
0 25–0 35	6 5– 9 0
0 15–0 25	4 0– 6 5
0 00–0 15	0 0– 4 0

TOTAL ACIDITY

REAGENTS

20 (a) *Hematin*.—Digest 0 5 g of hematin in 100 cc of cold neutral 95% alcohol

(b) *Gelatin soln*.—Soak 10 g of gelatine in H_2O at room temp for from 1 to 2 hours and then warm slightly not exceeding 50 °, to complete soln, add 25 cc of 95% alcohol and dilute If the gelatin soln is acid or alkaline neutralize with 0 1 *N* NaOH or 0 1 *N* acetic acid respectively using hematin indicator and dilute to 1 liter

(c) *Kaolin*.—Digest with HCl (1+9), wash until it complies with the tests given under **3**, dry and preserve in a tightly stoppered bottle

(d) 0 1 *N sodium hydroxide* [cf **II**, **17** (c)]

DETERMINATION[10]

21 Add 50 cc of the gelatin soln to 25 cc of the tanning liquor in a stoppered cylinder, dilute with H_2O to 250 cc add 15 g of the kaolin and shake vigorously Allow to settle for at least 15 min remove 30 cc of the supernatant liquid, dilute with 50 cc of H_2O and titrate with 0 1 *N* NaOH using the hematin indicator 1 cc of 0 1 *N* NaOH = 0 2% of acid, calculated as acetic in the liquor

RAW AND SPENT MATERIALS

(Under raw materials are included woods barks leaves etc)

MOISTURE IN SAMPLE AS RECEIVED

22 Cut or break up large pieces and mix the sample rapidly to avoid change in moisture content Dry as directed under **2**, a suitable weighed quantity dependent upon the physical condition and moisture content of the sample

PREPARATION OF SAMPLE

23 Dry the remainder of the sample at a temp not above 60 and grind to pass thru a 20 mesh sieve

MOISTURE IN PREPARED SAMPLE

24 Take 10 g of the sample prepared as directed under **23**, dry as directed under **2**, and calculate all results to an as received air dried or moisture-free basis as desired

25 EXTRACTION

(a) *Woods, barks, and spent materials* —Weigh such a quantity of the sample as will give an extract containing as nearly as possible 4 g of tannin per liter Transfer to a beaker and wet thoroly with hot H_2O Place a perforated porcelain plate in a tin lined Cu extractor of the general form shown in Fig 11, on the plate place a layer of cotton, and wet thoroly with H_2O Connect the extractor with an 800 cc Erlenmeyer flask (*G*) open the stop cock (*E*), and close the outlets (*C*) and (*D*) Pour into the extractor the material to be extracted, washing it into the extractor with hot H_2O Return the percolate thru the extractor until it is practically clear Place a layer of cotton on top of the material Close the stopcock (*E*), connect with an 800 cc Erlenmeyer flask containing about 650 cc of H_2O, connect (*D*) by a delivery tube with a liter graduated collecting flask, return the total percolate to the extractor, and connect by means of the metal cap (*B*) with a block tin condenser (*A*) in such a way that the condensate will drip upon the layer of cotton Boil the H_2O in the flask and collect 400–500 cc of percolate from the side tube (*D*) Open the stopcock (*E*) and close the side tube (*D*), add H_2O to the flask (*G*), if necessary, until it contains about 250 cc, and extract for 5 hours Remove the extract, add 200 cc of H_2O to the boiling flask, and continue the extraction for 9 hours Thruout the extraction heat at such a rate that approximately 330 cc of H_2O will be condensed per hour Combine all the extracts in the graduated liter flask in which the first percolate was received Heat to 80°, cool as directed under 1 (a), and dilute to the mark

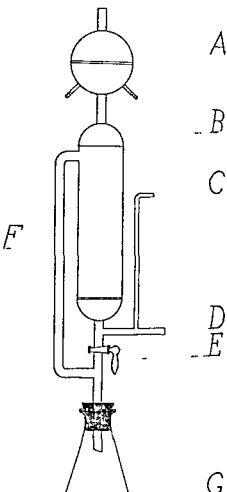

FIG 11 —METAL EXTRACTOR USED FOR EXTRACTING TANNING MATERIALS

(b) *Materials other than woods, barks, and spent materials* —Weigh as directed under 25 (a), a charge sufficient to give 2 liters of extract containing 4 g of tannin per liter Place in the extractor described under 25 (a), and digest for 1 hour with H_2O at room temp , at the end of which period start the extractor Keep stopcock (*E*) closed and collect entirely thru the side tube (*D*) 2 liters of percolate in approximately 7 hours Heat the percolate to 80°, cool as directed under 1 (a) and dilute to the mark

ANALYSIS OF THE EXTRACT

26

Proceed as directed under 2–8, inclusive With soins more dilute than specified, as is often the case with spent materials, reduce the quantity of hide powder used in the determination of nontannins in accordance with the concentration of the soln and the schedule given under 19

SELECTED REFERENCES

[1] The proceedings of the A O A C that deal with the early development of the methods of analysis of tanning materials will be found in Bureau of Chemistry Bul letins Nos 43, 47 49, 51, 56, 62, 67, 73, 81 and 90 These have been assembled in J Am Leather Chem Assoc 15, 12–127 (1920)
[2] J Am Leather Chem Assoc , 7, 288, 296 (1912)
[3] Ibid , 1, 32 (1906), 9, 412 (1914)
[4] Ibid , 10 282 (1915)
[5] The official hide powder of the American Leather Chemists Association is prepared only by the Standard Manufacturing Company, Ridgway, Pa
[6] J Am Leather Chem Assoc , 7, 292 (1912)
[7] Ibid , 23, 91 (1928)
[8] Ibid , 9, 36, 130 (1914)
[9] Ibid , 5, 5 (1910)
[10] Ibid , 3, 80 (1908), 4, 191 (1909)

XII PLANTS

1 DIRECTIONS FOR SAMPLING[1]—TENTATIVE

When more than one plant is sampled, include in the sample a sufficient number of plants to insure that it represents adequately the average composition of the entire lot of plants sampled This number cannot be stated definitely it will depend upon the variability in composition of the plants Details of the procedure must be determined by the purpose for which the sample is taken

2 PREPARATION OF SAMPLE[1]—TENTATIVE

(a) *For mineral constituents* —Thoroly remove all foreign matter from the material especially adhering soil or sand, avoiding excessive washing to prevent leaching, air dry as rapidly as possible to prevent decomposition or loss in weight by respiration grind, and preserve in tightly stoppered bottles If the results are to be expressed on the fresh weight basis, record the weights of the sample before and after air drying When determinations of Cu, Mn, Zn Fe Al, etc are to be made, take precautions to prevent contamination of the sample by dust during air drying and from the grinding and sieving machinery

(b) *For carbohydrates* —Thoroly remove all foreign matter and rapidly grind or chop the material into fine pieces Add the weighed sample to sufficient hot redistilled 95% alcohol to which sufficient precipitated $CaCO_3$ has been added to neutralize the acidity using sufficient alcohol so that the final concentration, allowing for the water content of the sample, will be approximately 80% Heat close to the boiling point on a steam or water bath for 30 min stirring frequently The samples may be stored until needed for analysis

3 MOISTURE—TENTATIVE

Determine as directed under XXVII, 2 or 6

4 ASH—TENTATIVE

Determine ash as directed under XXVII, 8

5 SAND AND SILICA—OFFICIAL

Ignite 10–50 g* of the substance in a flat bottomed Pt dish in a muffle, at a temp not exceeding dull redness until the residue is white or nearly so Dissolve in HCl (1+4) and transfer to a beaker or evaporating dish evaporate to dryness and heat on a water bath for 1 hour to render the SiO_2 insoluble Moisten the residue with 5–10 cc of HCl add about 50 cc of H_2O heat on a water bath for a few min , filter thru a hardened filter and wash thoroly To this filtrate add the filtrate and washings from the soluble SiO_2 determination and dilute to 200 cc Designate as A

Wash the residue from the filter into a Pt dish and boil for about 5 min with approximately 20 cc of a saturated soln of Na_2CO_3, add a few drops of a 10% NaOH soln, allow the mixture to settle and decant thru an ignited and weighed Gooch crucible Boil the residue in the dish with another 20 cc of the Na_2CO_3 sol and decant as before Repeat the process Transfer the Gooch cr and wash thoroly, first with hot H_2O then with a and finally hot H_2O until free from chlorides Dry the , and sand Confirm by microscopical examinat

[1] 10 g has been adopted as official

Determine the alkali soluble SiO_2 by the following procedure Combine the alka
filtrate and washings acidify with HCl evaporate to dryness and dehydrate by
heating to 110–120 for 2 hours Moisten the residue with 5–10 cc of HCl, add about
50 cc of H_2O heat on a water bath 10–15 min, filter thru an ashless filter or an
ignited and weighed Gooch crucible wash with hot H O, ignite and weigh as SiO_2

FERRIC AND ALUMINUM OXIDES—OFFICIAL

(Applicable to plant materials other than seeds)

IRON AND ALUMINUM—TENTATIVE

4

Take an aliquot of soln A containing approximately 10 mg of Fe and $AlPO_4$
Oxidize the Fe If the soln does not already contain an excess of phosphate, add to
an aliquot containing approximately 40 mg of Fe and $AlPO_4$ 0 5 g of $(NH_4)_2HPO_4$
stir until dissolved and make up to 50 cc with distilled H_2O Add a few drops of
thymol blue and then add NH_4OH until the soln just turns yellow Run in 0 5 cc
of HCl follow with 25 cc of 25% NH_4 acetate, and stir Let stand at room temp
until the precipitate settles (approximately 1 hour) Filter and wash 10 times with
hot 5% NH_4NO_3 soln Ignite and weigh as Fe and $AlPO_4$

Fuse the ignited precipitate in a Fe crucible with about 4 g of a mixture of equal
parts of Na_2CO_3 and K_2CO_3 When the fusion is complete allow the crucible to cool
add 5 cc of H_2SO_4 and heat until copious fumes of SO_3 are given off Cool transfer
to a flask add H_2O and digest until the soln is clear Reduce the Fe with Zn cool,
and titrate with 0 1 N KMnO4 soln (cf XXXVII, 58) Report as percentage of
Fe_2O_3 Calculate the oxide to phosphate and subtract from total Fe and AlPO4
This gives the $AlPO_4$ Calculate to and report as Al_2O_3

MICRO METHOD FOR IRON ONLY—TENTATIVE

7

Place an aliquot of soln A containing approximately 0 2 mg of Fe in a 50 cc volu
metric flask Oxidize the Fe by boiling with a few drops of HNO_3 and cool to
about 35 cc of HCl and 0 3 cc of HNO_3 and cool to about 15° Now add 10 cc of
20% KCNS soln fill to the mark with distilled H_2O and compare the intensity of
color with that of a standard containing somewhere near the same amount of Fe as
the sample and similarly prepared If the quantity of Fe is extremely small, extract
both the sample and standard with equal parts of amyl alcohol and ether and com
pare the extracts in a colorimeter Calculate the amount of Fe pre ent

MICRO METHOD FOR ALUMINUM ONLY—TENTATIVE

8

Take an aliquot of soln A containing approximately 0 0 mg of Al Oxidize the
Fe by boiling with a few drops of HNO_3 and transfer to a conical centrifuge tube of
about 25 cc capacity with marks at 15 20 and 25 cc If the quantity of Fe is very
small add 1 cc of a soln equivalent to about 1 mg of iron or if the quantity of phos
phate present is small add about 0 1 g of $(NH_4)_2HPO_4$ to insure complete precipita
tion of the Fe and Al Dilute the contents to about 15 cc with distilled H_2O neu
tralize with NH_4OH using a drop of dilute methyl red as indicator and add 1 cc of
a saturated soln of NH_4 acetate Place the tube in a water bath until the precipitate
begins to settle (usually about 10 min) centrifugalize decant and discard the
supernatant liquid Dissolve the precipitate in 1 cc (1 cc approximately 0 5 N HCl with
warming when necessary and dilute to 15 cc (1 cc approximately 0 5 N HCl with
an 1 cc of 0 5 N NaOH (special Al free) wash down the sides and fill to the 25 cc
mark Let stand 1 hour and centrifugalize The precipitate contains the
Fe and the soln the Al

Transfer to a 50 cc volumetric flask as large an aliquot as can be drawn off Add H_2O to make about 20 cc, a small piece of litmus paper, and finally HCl (1+9) until the litmus paper just turns red Determine the Al as follows Add 5 cc of 5 *N* NH_4-acetate, 5 cc of 1 5 *N* HCl, and 2 cc of 0 1% of the dye Aluminon (ammonium salt of aurintricarboxylic acid) and place in a water bath at about 80 for 10 min Add 5 cc of 5 *N* NH_4Cl, cool to room temp , add 5 cc of 3 2 *N* $(NH_4)_2CO_3$ while shaking gently, fill to the mark with distilled H_2O, and mix At this point the reactions should be PH 7 1–7 3 and the red color of a blank should disappear in about 15 min (The exact concentration of the reagents is not important, but the final pH is, and the amount of NH_4CO_3 necessary to bring the soln to the above pH should be determined by neutralizing similar solns without adding the dye) Simultaneously with the above procedure run a standard (or standards if necessary) containing a given quantity of Al After allowing the mixture to stand for 20 min for the excess dye to decolorize, compare the color intensities and read the amount of Al from a curve plotted as described in the following paragraph

If only a small number of determinations are to be made, prepare 4 standards containing 0 01, 0 03, 0 05 and 0 07 mg of Al, respectively, and run these with the samples Compare all these solns with the standard containing 0 03 mg of Al and calculate the results to a colorimeter reading of 30 for this standard Arbitrarily give 0 005 mg of Al a reading of 100 and with this and the 4 readings on the standards plot a curve * Read the quantity of Al in each sample from this curve If a large number of determinations are to be made extending over a period of time, it is advisable to make determinations on several series of standards and plot a curve from the average of these results It is then necessary to run only 1 standard each time determinations are to be made, and the results can be read from the curve Blanks must be run on both the Fe and Al determinations as nearly all the reagents contain traces of these elements

MANGANESE CALCIUM AND MAGNESIUM—OFFICIAL†

(Applicable to plant materials other than seeds)

9 CALCIUM—OFFICIAL FIRST ACTION

Transfer an aliquot of soln A, under 5, corresponding to 0 5–2 g of ash, to a 300 cc beaker and dilute to 200 cc Add a few drops of alizarine or methyl orange indicator and make slightly alkaline with NH_4OH (1+4) Add HCl (1+4) until the soln is just faintly acid, and then add 10 cc of 0 5 *N* HCl and 10 cc of a 2 5% oxalic acid soln Boil the mixture and add, with constant stirring, 15 cc of a saturated soln of $(NH_4)_2$ oxalate Continue to heat until the precipitate becomes granular Cool, add, with constant stirring, 8 cc of a 20% Na acetate soln, and allow to stand 12 hours Filter and wash with hot H_2O until free from chlorides Break the point of the filter with a stirring rod and wash the precipitate into the beaker with hot H_2O Add 10 cc of H_2SO_4 (1+1), heat nearly to boiling, and titrate with 0 1 *N* $KMnO_4$ soln Finally add the filter to the soln and complete the titration 1 cc of 0 1 *N* $KMnO_4$ = 0 002[illegible] g of CaO Report as percentage of CaO

Or proceed as follows To the aliquot add 10 cc of a saturated soln of NH_4-oxalate and a drop of methyl red Almost neutralize with NH_4OH, and boil until the precipitate is coarsely granular Cool, add a few more drops of methyl red and then NH_4OH (1+4) until the color is a faint pink (pH 5 0), and allow to stand at least 4 hours Filter, and wash, etc , as directed above

* See original article *J Am Chem Soc* 51 2729 (1929)
† See note 7 p xvii

Micro Method—Tentative

10 REAGENTS

(a) *Potassium permanganate* —0 02 *N*
(b) *Ammonium oxalate* —Saturated soln
(c) *Glacial acetic acid* —(1 + 1)
(d) *Ammonium hydroxide* —(1 + 1)
(e) *Ammonium hydroxide* —(1 + 49)
(f) *Sulfuric acid* —(1 + 4)

11 APPARATUS

(a) *Centrifuge tubes* —Conical tipped, about 15 *cm* long and of about 18 mm or 20 mm inside diameter
(b) *Suction device* —With tip as shown in diagram
(c) *Centrifuge* —About 2500 r p m

12 DETERMINATION

Ignite 2 g of the substance in a small crucible in a muffle at dull red heat Dissolve the ash in HCl (1 + 1) and transfer to a 100 cc beaker Add 5 cc of HCl and evaporate to dryness on the steam bath to dehydrate the SiO_2 Moisten the residue with 5 cc of HCl, add about 50 cc of distilled H_2O, heat for a few minutes on the steam bath, transfer to a 100 cc volumetric flask, cool quickly to room temp , make to volume, shake, and filter discarding the first portion of the filtrate Pipet a 15 cc aliquot into a conical tipped centrifuge tube containing 2 cc of saturated (NH_4) oxalate soln and 2 drops of 0 05% methyl red Add 2 cc of dilute acetic acid rotating the tube to mix its contents thoroly Add while intermittently rotating the tube NH_4OH (1 + 4) until the soln is faintly alkaline, after which add a few drops of dilute acetic acid with a dropper until the color is adjusted to a faint pink (pH 5 0) (It is important at this point to rotate the tube so that the last bit of liquid in the conical tip is the color required) Allow the mixture to stand at least 4 hours and whirl the tube in the centrifuge for 15 min (The precipitate should then be in a firm lump in the tip of the tube) Remove the supernatant liquid by means of the suction device shown in the diagram taking care not to disturb the precipitate Wash the precipitate by adding 2 cc of Reagent (e) rotating the tube to break up the precipitate (It may be necessary to jar the tube sharply) Return the tube to the centrifuge for 10 min and again remove the supernatant liquid and wash with the reagent as before Repeat this operation until the precipitate has been washed 3 times When the supernatant liquid has been removed after the final centrifugalizing add 2 cc of Reagent (f) to the tube break up the precipitate as before heat on the steam bath to between 80 and 90 and titrate in the tube with 0 02 *N* $KMnO_4$ rotating the liquid during the titration to attain a

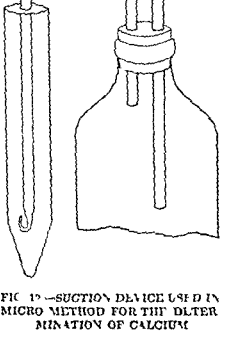

FIG 12 —SUCTION DEVICE USED IN MICRO METHOD FOR THE DETERMINATION OF CALCIUM

Transfer to a 50 cc volumetric flask as large an aliquot as can be drawn off Add H_2O to make about 20 cc, a small piece of litmus paper, and finally HCl (1+9) until the litmus paper just turns red Determine the Al as follows Add 5 cc of 5 *N* NH_4-acetate, 5 cc of 1 5 *N* HCl, and 2 cc of 0 1% of the dye Aluminon (ammonium salt of aurintricarboxylic acid) and place in a water bath at about 80° for 10 min Add 5 cc of 5 *N* NH_4Cl, cool to room temp , add 5 cc of 3 2 *N* $(NH_4)_2CO_3$ while shaking gently, fill to the mark with distilled H_2O, and mix At this point the reactions should be PH 7 1–7 3 and the red color of a blank should disappear in about 15 min (The exact concentration of the reagents is not important, but the final pH is, and the amount of NH_4CO_3 necessary to bring the soln to the above pH should be determined by neutralizing similar solns without adding the dye) Simultaneously with the above procedure run a standard (or standards if necessary) containing a given quantity of Al After allowing the mixture to stand for 20 min for the excess dye to decolorize, compare the color intensities and read the amount of Al from a curve plotted as described in the following paragraph

If only a small number of determinations are to be made, prepare 4 standards containing 0 01, 0 03, 0 05 and 0 07 mg of Al, respectively, and run these with the samples Compare all these solns with the standard containing 0 03 mg of Al and calculate the results to a colorimeter reading of 30 for this standard Arbitrarily give 0 005 mg of Al a reading of 100 and with this and the 4 readings on the standards plot a curve * Read the quantity of Al in each sample from this curve If a large number of determinations are to be made extending over a period of time, it is advisable to make determinations on several series of standards and plot a curve from the average of these results It is then necessary to run only 1 standard each time determinations are to be made, and the results can be read from the curve Blanks must be run on both the Fe and Al determinations as nearly all the reagents contain traces of these elements

MANGANESE CALCIUM AND MAGNESIUM—OFFICIAL†

(Applicable to plant materials other than seeds)

9 **CALCIUM‡—OFFICIAL FIRST ACTION**

Transfer an aliquot of soln A, under 5, corresponding to 0 5–2 g of ash, to a 300 cc beaker and dilute to 200 cc Add a few drops of alizarine or methyl orange indicator and make slightly alkaline with NH_4OH (1+4) Add HCl (1+4) until the soln is just faintly acid, and then add 10 cc of 0 5 *N* HCl and 10 cc of a 2 5% oxalic acid soln Boil the mixture and add, with constant stirring, 15 cc of a saturated soln of $(NH_4)_2$-oxalate Continue to heat until the precipitate becomes granular Cool, add, with constant stirring 8 cc of a 20% Na acetate soln, and allow to stand 12 hours Filter, and wash with hot H_2O until free from chlorides Break the point of the filter with a stirring rod and wash the precipitate into the beaker with hot H O Add 10 cc of H_2SO_4 (1+1), heat nearly to boiling, and titrate with 0 1 *N* $KMnO_4$ soln Finally add the filter to the soln and complete the titration 1 cc of 0 1 *N* $KMnO_4$ = 0 0028 g of CaO Report as percentage of CaO

Or proceed as follows To the aliquot add 10 cc of a saturated soln of NH_4-oxalate and a drop of methyl red Almost neutralize with NH_2OH, and boil until the precipitate is coarsely granular Cool, add a few more drops of methyl red and then NH_4OH (1+4) until the color is a faint pink (pH 5 0), and allow to stand at least 4 hours Filter, and wash, etc , as directed above

* See original article *J Am Chem Soc* 51 2729 (1929)
† See note 7 p xvii

proper end point If the tube cools below 60° during the addition of the $KMnO_4$ reheat it in the steam bath for a few minutes and complete the titration Run a blank on an identical amount of dilute H_2SO_4 in a similar tube heated to the same temp to determine the quantity of 0 02 *N* $KMnO_4$ necessary to give the color of the end point Subtract this value from the buret reading 1 cc of 0 02 *N* $KMnO_4$ = 0 0004 g of Ca Report as percentage of Ca

13 MAGNESIUM[7]—OFFICIAL FIRST ACTION

To the combined filtrate and washings from the Ca determination (9) add 40 cc of HNO_3 and evaporate to dryness to decompose the NH_4 salts Take up with HCl (1+4) and make to a volume of about 100 cc with H_2O Add 5 cc of a 10% Na citrate soln and 10 cc of a 10% Na_2H-PO_4 soln, or enough to precipitate all the Mg Add NH_4OH (1+4), with constant stirring until the soln is faintly alkaline then add about 25 cc of NH_4OH and allow to stand in a cool place overnight Filter and wash with NH_4OH (1+9) Dissolve the precipitate in HCl (1+4) and reprecipitate as before Allow to stand several hours filter wash free of chlorides with the NH_4OH (1+9), ignite, and weigh as $Mg_2P_2O_7$ Calculate and report the result as percentage of MgO

14 MANGANESE[8]—OFFICIAL

To an aliquot of soln A, under 5, representing about 0 2–0 5 g of ash, add 15 cc of concentrated H_2SO_4 and evaporate to about 30 cc Add 5–10 cc of HNO_3 and continue the evaporation (It is neither necessary nor advisable to evaporate until dense fumes appear, since the $Fe(SO_4)_3$ then dissolves with difficulty HNO_3 may be present, but not HCl) Add H_2O, a little at a time, heat until the Fe salts have dis

in hot H_2O Add 5 cc of a saturated soln of $Ba(OH)_2$ heat to boiling, allow to settle a few min , and determine whether or not the precipitation is complete by the addition of more of the $Ba(OH)_2$ soln to a little of the clear liquid When no further precipitate is produced, filter and wash thoroly with hot H_2O Heat the filtrate to boiling and add NH_4OH (1+4) and a 10% $(NH_4)_2CO_3$ soln to complete the precipitation of the Ba Ca, etc Let stand a short time on a water bath, filter, and wash the precipitate thoroly with hot H_2O Evaporate the filtrate and washings to dryness expel NH_4 salts by heating below redness, treat with a little hot H_2O, and add a few drops of the dilute NH_4OH, 1 or 2 drops of the $(NH_4)_2CO_3$ soln, and a few drops of a saturated soln of NH_4 oxalate Let stand for a few min on a water bath and set aside for a few hours Filter, evaporate to complete dryness on a water bath, and heat at a temp not exceeding dull redness until all NH_4 salts are expelled and the residue is nearly or quite white Dissolve in a minimum quantity of H_2O filter into a weighed Pt dish add a few drops of HCl, evaporate to dryness on a water bath, heat at a temp not exceeding dull redness cool in a desiccator and weigh as KCl plus NaCl Repeat the heating until constant weight is obtained Dissolve in a small quantity of H_2O, if any residue remains, repeat the separation until the residue of KCl and NaCl is entirely soluble Dissolve the residue with H_2O add an excess of Pt soln [cf II, 41 (b)] and proceed as directed under II, 46

Method II—Official

16 Proceed as directed under 15 thru Let stand a short time on a water bath, ' the point at which the Ba, Ca, etc have been precipitated with NH_4OH and $(NH_4)_2CO_3$, and then proceed as follows

Filter into a beaker add 1 or 2 drops of HCl (1+4) and 1 cc of $(NH_4)_2SO_4$ soln (75 g to 1 liter) digest several hours on a water bath and filter into a weighed Pt dish Evaporate to dryness heat to full redness and add 1 g of powdered $(Na_2)_2CO_3$ Heat to expel excess of $(NH_4)_2CO_3$ cool, and weigh the sulfates of Na and K De termine K as directed under II, 43 (a)

COPPER—OFFICIAL FIRST ACTION

REAGENTS

17 (a) *Potassium ethyl xanthate* —0 1% water soln prepared fresh each time it is used

(b) *Standard copper sulfate* —Dissolve 0 3928 g of pure $CuSO_4\ 5H_2O$ in H_2O di lute in a volumetric flask to 1000 cc, and mix 1 cc = 0 0001 g of Cu

(c) *Filter paper pulp* —Moisten and tear a good grade of sheet filter paper into bits and place in a porcelain dish of the proper size Add while stirring with a glass rod enough cold HCl to disintegrate the paper and reduce the mass to a mushy consistency Transfer the pulp to a large Buchner funnel and wash free of acid using suction Transfer the washed pulp into a clean glass bottle and add H_2O to make a thick pulp suitable for making a pad in a Caldwell crucible (The precipitates of Cu and Zn obtained in these methods can be readily filtered and washed upon pads made with this filter paper pulp by the use of the suction pump)

(d) *Hydrochloric acid* —0 2o *M* soln

DETERMINATION

18 Ash 100–500 g of the finely divided air dried plant material in SiO_2 dishes with a small flame but do not allow the plant material to burn with a blaze After the vola tile matter has been dispelled complete the ashing in a muffle furnace maintained at the temp of a faint red glow Hasten the ashing process by removing the dishes from the muffle at intervals and breaking up the lumps with a Pt stirring rod After

the C has been oxidized as completely as possible, cool the dish, moisten the ash with H_2O, wash into a 250 cc beaker, and cover with a watch glass Decompose the ash with HCl (1+1) introduced thru the lip of the beaker beneath the watch glass by means of a pipet After effervescence has ceased, rinse the watch glass into the beaker, filter the insoluble residue out on a Büchner funnel, and wash free of chlorides If the insoluble residue contains undecomposed particles of C, transfer it into a SiO_2 dish and reignite in the muffle furnace until all particles of C are decomposed and a light colored ash remains Redigest the ash on a hot water bath with 15 cc of HCl (1+1), filter, and wash free of chlorides Combine the filtrates in a clean porcelain dish, evaporate to dryness, and bake at 110° until the HCl is expelled Moisten the dry residue with 10 cc of HCl (1+1) and digest, with stirring, for 10 min Dilute with hot H_2O, filter out the SiO_2, wash free of chlorides, combine with the insoluble residue, ignite, and weigh Make the filtrate to about 250 cc, heat to near the boiling point, and pass a slow stream of H_2S thru the soln for 15 min Rinse the H_2S delivery tube into the flask, tightly stopper, and set aside until the precipitate settles and the supernatant soln is clear Filter the CuS off on a pad of paper pulp and wash with Reagent (d) saturated with H_2S, ignite in a porcelain crucible and dissolve the CuO in a few drops of HNO_3 (1+9) and one drop of HCl (1+9) Filter the soln and wash the filter paper clean Evaporate the soln to dryness in a porcelain dish 3 times with the addition of a few drops of HNO_3 (1+9) and take up with a very small drop of HNO_3 (1+9) delivered from a stirring rod having a sharp point Make to a volume of 50 cc, and transfer an aliquot of 5 cc to a Nessler tube containing 10 cc of Reagent (a) Mix the solns and dilute to 25 cc Transfer 10 cc of Reagent (a) to a second Nessler tube, dilute to a volume of about 15 cc, and add the standard Cu soln (b) dropwise with thoro mixing with a glass stirring rod until the color in the standard tube apparently matches the color in the tube containing the sample Make the volume and the final adjustment of the color of the standard in the tube containing it Record the number of cc of the standard Cu soln required and calculate the percentage of Cu

To the filtrate from the CuS add 5 cc of HNO_3 (1+1) and boil for 10 min to oxidize the remaining metals Cool the soln and make to a convenient volume (250 cc) From this stock soln take suitable aliquots for the determination of Zn or other elements contained in the ash of the sample

ZINC—OFFICIAL FIRST ACTION

19 REAGENTS

(a) *Potassium ferrocyanide*.—2 g per 100 cc of freshly prepared soln

(b) *Zinc sulfate*.—Dissolve 1 g of C P Zn in H_2SO_4 and dilute to 1000 cc 1 cc = 0 001 g of Zn

20 DETERMINATION

Transfer an aliquot equivalent to 25 g of plant material from the stock soln (61 trate from the Cu determination—last paragraph under 18) to a 250 cc Erlenmeyer flask and add NH_4OH in slight excess Dissolve the precipitate in a slight excess of pure acetic acid, saturate the soln with H_2S, and set aside for several hours for the precipitate to settle [The acidity of the soln must be kept between pH 2 and pH 3, and the presence of a citrate helps to prevent the precipitation of Fe and Mn Hence at this point add about 2 g of citric acid, ammonia until neutral to methyl orange and then 10 cc of a formic soln (containing in 100 cc of the mixture, 3 cc of NH_4OH 20 cc of 90% formic acid, and 25 g of $(NH_4)_2SO_4$)] Filter on a pad of paper pulp and wash with a dilute soln of acetic acid containing NH_4 acetate saturated with H_2S Ignite the pad of paper pulp and precipitate in a porcelain crucible, cool dissolve

the residue in a few drops of HCl (1+9) and warm on the hot water bath Transfer to a 100 cc beaker and add NH_4OH in slight excess Heat on the water bath for 5 min , filter, and wash the precipitate Add acetic acid in slight excess to the filtrate and saturate the hot soln with H_2S, stopper tightly and set aside several hours in a warm place for the precipitate to settle filter, wash as previously described and ignite in a porcelain crucible Dissolve the ignited residue of ZnO in 10 cc of 0 1 N H_2SO_4, make to a volume of 50 cc, and mix Transfer an aliquot of 5 cc to a 50 cc Nessler tube containing 5 cc of Reagent (a) Dilute to 50 cc, mix with a stirring rod, and let stand 5–10 min To another Nessler tube containing 5 cc of Reagent (a) diluted to about 40 cc, add dropwise with stirring the standard soln (b) until the turbidity in the standard matches the turbidity of the sample From the number of cc of the Zn standard required calculate the percentage of Zn contained in the sample

ARSENIC—TENTATIVE

21 PREPARATION OF SOLUTION

Prepare as directed under XXIX, 3

22 DETERMINATION

Determine the As as directed under XXIX, 4, or take an aliquot and determine as directed under VI, 13, beginning with "add 3 cc of H_2SO_4"

SULFUR

Sodium Peroxide Method[12]*—Official*

23 PREPARATION OF SOLUTION

Place 1 5–2 5 g of material in a Ni crucible of about 100 cc capacity and add 5 g of anhydrous Na_2CO_3 Mix thoroly using a Ni or Pt rod and moisten with approximately 2 cc of H_2O Add Na_2O_2, approximately 0 5 g at a time thoroly mixing the charge after each addition, and continue until the mixture becomes nearly dry and quite granular (Usually about 5 g of Na_2O_2 is required) Place the crucible over a low alcohol or other S free flame and heat carefully, with occasional stirring, until the contents are fused (If the material ignites the determination is worthless) After fusion, remove the crucible allow to cool somewhat and cover the hardened mass with more of the Na_2O_2 to a depth of about 0 5 cm Heat gradually and finally with full flame until fusion again takes place rotating the crucible from time to time in order to bring any particles adhering to the sides into contact with the oxidizing material Continue the heating for 10 min after fusion is complete Cool somewhat, place the warm crucible and contents in a 600 cc beaker, and carefully add about 100 cc of H_2O After the initial violent action has ceased, wash the material out of the crucible make slightly acid with HCl (adding small portions at a time), transfer to a 500 cc flask cool and dilute to volume Filter and determine sulfates in an aliquot of the filtrate as directed under 25

24 REAGENT

Barium chloride —10% soln

25 DETERMINATION

Heat to boiling and add slowly in small quantities a 10% $BaCl_2$ soln until no further precipitate is formed Continue the boiling for about 5 min and allow to stand for 5 hours or longer in a warm place Decant the liquid thru an ashless filter or an ignited and weighed Gooch crucible treat the precipitate with 15–20 cc of boiling H_2O transfer to the filter and wash with boiling H_2O until the filtrate is free from

chlorides Dry the precipitate and filter, ignite, and weigh as $BaSO_4$ Multiply the result by the factor 0 13736 and report as percentage of S

Magnesium Nitrate Method[11]—Official, First Action

26 PREPARATION OF SOLUTION

Weigh a quantity of the sample sufficient to give a precipitate of not less than 30 mg of $BaSO_4$ into a large porcelain or Sillimanite crucible Add 7 5 cc of $Mg(NO_3)_2$ soln {II, 5(e)}, taking care that all the material is brought in contact with the soln and heat on an electric hot plate (180°) until no further action takes place Transfer the crucible while hot to an electric muffle and allow it to remain at low heat (muffle must not show any red) until the charge is thoroly oxidized No black particles should remain (It may be necessary to break up the charge and return to the muf fle) Remove the crucible from the muffle and allow to cool Add H_2O, then HCl in excess Bring the soln to a boil filter, and wash thoroly If preferred, transfer the soln to a 250 cc volumetric flask before filtering and make to the mark with H_2O

27 DETERMINATION

Dilute the entire filtered soln, prepared as directed under 26, to 200 cc or take an aliquot of 100 cc of the measured volume, make to 200 cc, and proceed as directed under 25

PHOSPHORUS[11]

28 *Method I—Official, first action*

Take 100 cc of the original soln prepared as directed under 26, or evaporate the filtrate and washings from the S determination, 27, to 75 cc and proceed as directed under II, 7 or 10

II Micro Method[12]—Tentative

29 REAGENTS

(a) *Potassium dihydrogen phosphate standard*—Dissolve 0 1394 g of pure dry KH_2PO_4 in distilled H_2O and make up to a liter 50 cc of this soln diluted to 200 cc gives a standard of which 2 cc = 0 05 mg of P

(b) *Ammonium molybdate*—Dissolve 25 g of NH_4 molybdate in 300 cc of H_2O Dilute 75 cc of H_2SO_4 to 200 cc and add to the NH_4 molybdate soln

(c) *Hydroquinone*—Dissolve 0 5 g of hydroquinone in 100 cc of distilled H_2O and add one drop of concentrated H_2SO_4 to retard oxidation

(d) *Sodium sulfite*—Dissolve 200 g of Na_2SO_3 in distilled H_2O make up to a liter, and filter Either keep this soln well stoppered or make it up fresh each time

(e) *Magnesium nitrate*—Dissolve 160 g of MgO in HNO_3 (1+1), avoiding an ex cess of the acid, add a little MgO in excess, boil, filter from the excess MgO, Fe O, etc , and dilute to 1 liter

30 PREPARATION OF SOLUTION

To 1 or 2 g of the substance in a small Sillimanite crucible add 1 cc of the $Mg(NO_3)_2$ soln and place on the steam bath After a few min cautiously add a few drops of HCl, taking care that the formation of gas bubbles does not push portions of the sample over the edge of the crucible Make 2 or 3 further additions of a few drops of HCl while the sample is on the bath so that as it approaches dryness there is a tendency for it to char If the contents of the crucible become so viscous that no further drying may be obtained on the bath, complete the drying on a hot plate

put on a crucible cover, transfer to a cold muffle, and ignite at dull red heat for 6 hours or until an even grey ash is obtained It may be necessary to cool the crucible, dissolve the ash in a little H_2O or alcoholic glycerol, evaporate to dryness, and return uncovered to the muffle for another 4 or 5 hours Cool take up with HCl (1+4), and transfer to a 100 cc beaker Add 5 cc of HCl and evaporate to dryness on the steam bath to dehydrate the SiO_2 Moisten the residue with 2 cc of HCl, add about 50 cc of distilled H_2O heat for a few minutes on the bath, transfer to a 100 cc volumetric flask, cool immediately make to volume, and filter discarding the first portion of the filtrate

31 DETERMINATION

To a 5 cc aliquot of the filtrate in a 10 cc volumetric flask add 1 cc of NH_4 molybdate rotate the flask to mix and allow to stand a few moments Add 1 cc of hydroquinone again rotate the flask, and add 1 cc of Na_2SO_3 These last 3 additions may be made with a Mohr pipet Make to volume with distilled H O stopper the mouth of the flask with the thumb or forefinger, and shake to mix the contents thoroly Allow to stand 30 min and compare immediately in a colorimeter with 2 cc of the standard KH_2PO_4 soln treated simultaneously and in an identical manner With either the unknown or standard set at 25 0 mm readings within 10 mm (i e a range of 20 mm) are accurate If the concentration of P in the unknown set is outside this range it may be brought nearer to that of the standard by diluting the filtrate, ashing a smaller or larger sample making the filtrate to a smaller or larger volume, or using a smaller aliquot Report as percentage of P

CHLORINE—OFFICIAL

32 PREPARATION OF SOLUTION

Moisten 5 g of the substance in a Pt dish with 20 cc of a 5% Na_2CO_3 soln evaporate to dryness and ignite as thoroly as possible at a temp not exceeding dull red ness Extract with hot H O, filter and wash Return the residue to the Pt dish and ignite to an ash dissolve in HNO_3 (1+4) filter from any insoluble residue wash thoroly, and add this soln to the H_2O extract

33 *I Gravimetric Method*

To the soln prepared as directed in 32 add a 10% $AgNO_3$ soln avoiding more than a slight excess Heat to boiling protect from the light, and allow to stand until the precipitate is granular Filter on a weighed Gooch crucible previously heated to 110–150 and wash with hot H_2O, testing the filtrate to prove excess of $AgNO_3$ Dry the AgCl at 110–150 cool and weigh Report as percentage of Cl

II Volumetric Method[12]

34 REAGENTS

(a) *Silver nitrate* —Adjust to exact 0 1 *N* strength by standardizing against a 0 1 *N* NaCl soln containing 5 846 g of pure NaCl per liter

(b) *Ammonium or potassium thiocyanate* —0 1 *N* Adjust by titrating against the 0 1 *N* $AgNO_3$

(c) *Ferric indicator* —A saturated soln of ferric ammonium alum

(d) *Nitric acid* —Free from lower oxides of N by diluting the usual pure acid with about ¼ volume of H_2O and boiling until perfectly colorless

35 DETERMINATION

To the soln prepared as directed under 32, add a known volume of the 0 1 *N* $AgNO_3$ in slight excess Stir well, filter and wash the AgCl precipitate thoroly To

the combined filtrate and washings add 5 cc of the ferric indicator and a few cc of the HNO_3 and titrate the excess of Ag with the 0 1 *N* thiocyanate until a permanent light brown color appears From the number of cc of 0 1 *N* $AgNO_3$ used, calculate the quantity of Cl 1 cc of 0 1 *N* $AgNO_3$ = 0 00355 g of Cl

TOTAL NITROGEN

36 Determine as directed under II, 27

SUGARS[11]—TENTATIVE

PREPARATION OF SOLUTION

37 *Extraction* —Prepare the sample as described under 2(b) Pour the alcoholic soln thru a filter paper or extraction thimble, catching the filtrate in a volumetric flask Transfer the insoluble material to a beaker, cover with 80% alcohol, warm on a steam bath for 1 hour, allow to cool, and again pour the alcoholic soln thru the same filter If the second filtrate is highly colored, repeat the extraction Transfer the residue to the filter allow to drain and dry Grind the residue so that all the particles will pass thru a 1 mm sieve then transfer it to an extraction thimble and extract for 12 hours in a Soxhlet apparatus with 80% alcohol Dry the residue and save for the starch determination Combine the alcoholic filtrates and make to volume at a definite temp with 80% alcohol

Clearing —Place an aliquot of the alcoholic extract in a beaker on the steam bath and drive off the alcohol Avoid evaporation to dryness by adding H_2O if necessary When the odor of alcohol has disappeared from the sample, add about 100 cc of distilled H_2O and heat to 80° to soften gummy precipitates and break up insoluble masses Cool to room temp and proceed as directed under (a) or (b)

(a) Transfer the soln to a volumetric flask and rinse the beaker thoroly with H_2O, adding the rinsings to the contents of the flask Add enough saturated neutral Pb acetate to produce a flocculent precipitate, shake thoroly, and allow to stand 15 min Test the supernatant liquid with a few drops of saturated Pb-acetate If more precipitate forms shake and allow to stand again if no further precipitate forms, dilute to the mark with H_2O, mix thoroly, and filter thru a dry filter Add sufficient solid Na-oxalate to the filtrate to precipitate all the Pb, and refilter thru a dry paper Test the filtrate for the presence of Pb with a little solid Na oxalate

(b) Add double the minimum amount of saturated neutral Pb-acetate soln that is required to cause complete precipitation as found by testing a portion of the supernatant liquid with a few drops of dilute Na-oxalate soln After allowing the mixture to stand a few min only, filter immediately into a beaker to which has been added an estimated excess of Na oxalate crystals Allow the Pb precipitate to drain on the filter and wash with cold H_2O until the filtrate no longer gives a precipitate in the oxalate soln Excess of oxalate must be assured by testing with a drop of dilute Pb-acetate soln Filter off and wash the precipitated Pb-oxalate, catching the filtrate and washings in a volumetric flask Dilute to the mark with H_2O and mix

REDUCING SUGARS—TENTATIVE

I Munson and Walker General Method

39 Proceed as directed under XXXIV, 37

II Qutsumbing and Thomas Method[15]

REAGENTS

40 (a) *Copper sulfate soln* —Wash crystals of C P $CuSO_4 \cdot 5H_2O$ free from dust, etc with distilled H_2O, dissolve in hot H_2O to make a saturated soln, and filter Deter

mine the Cu electrolytically and dilute the soln so that 25 cc of it will contain 525 mg of Cu or 41 2 g of $CuSO_4$ pentahydrate in 500 cc of soln

(b) *Alkaline tartrate soln* —Prepare a saturated soln of NaOH (purified by alcohol) and let stand until the insoluble carbonates and other impurities have settled out several days Siphon off the clear soln and establish its alkalinity by titration with standard acid Dissolve 173 g of highest purity Rochelle salts in H_2O in a 500 cc graduated flask and add the calculated quantity of NaOH soln so that 500 cc of this alkaline tartrate soln will contain exactly 65 g of NaOH Make to the mark with H_2O

41 PRECIPITATION OF CUPROUS OXIDE

Measure exactly 25 cc each of the $CuSO_4$ and alkaline tartrate solns into a 400 cc Pyrex or Bohemian glass beaker, the diameter of which is about 9 cm Add 50 cc of sugar soln containing preferably 50–150 mg of sugar Cover the beaker with a watch glass and place the beaker in a water bath which is maintained at 80° After exactly 30 min digestion filter the Cu_2O by suction thru a mat of asbestos in a Gooch crucible Wash the precipitate with H_2O Determine the Cu by one of the methods below Calculate the weight of sugar from the tables of Quisumbing and Thomas (XLII, 17)

DETERMINATION OF REDUCED COPPER

42 *I Direct Weighing of Cuprous Oxide*

Proceed as directed under XXXIV, 39

43 *II Volumetric Permanganate Method*

(a) Proceed as directed under XXXIV, 42

(b) Filter and wash the Cu_2O as directed in 41 Transfer the asbestos film to the beaker suspend in H_2O and heat the precipitate and asbestos thoroly Rinse the crucible and the lip of the beaker with 10 cc of a soln made by dissolving 240 g of crystalline ferric ammonium sulfate and 200 cc of H_2SO_4 in H_2O to 1 liter (Note Cool the diluted H_2SO_4 before dissolving the alum) Receive the rinsings in the beaker containing the precipitate Wash the crucible and sides of the beaker with boiling H_2O catching the washings in the beaker Stir until all the Cu is dissolved A green soln is obtained Titrate at once with continual stirring with $KMnO_4$ soln until the pink due to the $KMnO_4$ persists for about 10–15 seconds 1 cc of 0 05 *N* $KMnO_4$ = 0 0031755 g of Cu Standardize the $KMnO_4$ as follows Dry overnight about 0 66 g of Na oxalate (pure) in a weighing tube in an oven at 100° and carefully weigh off 3 samples of 0 10–0 15 g each Dissolve each sample in 100 cc of H_2O, add 5 cc of H_2SO_4 (1+1) warm to 70 , and titrate the $KMnO_4$ against this soln Take the average of the 3 titrations 1 cc of 0 05 *N* $KMnO_4$ = 0 0033 g of Na oxalate

44 *III Electrolytic Deposition from Sulfuric and Nitric Acid Solutions*

Proceed as directed under XXXIV, 43

45 SUCROSE—TENTATIVE

(a) *Hydrochloric acid inversion* —Proceed as directed under XXVII, 22

(b) When glucosides which are easily hydrolyzed by HCl are present sucrose may be inverted by invertase The preparation and use of invertase are described under XXXIV, 21 The quantity of invertase to be used depends on its activity Avoid a large excess because it causes difficulty in the filtration of the reduced Cu

XV

by (*G*) Connect *H* with an aspirator Many analysts prefer to replace the bulb *F* by two U tubes filled with sifted soda lime

DETERMINATION

4 Place 0 5–2 g of the sample, the quantity depending upon the percentage of CO_2 present, in flask *A*, which must be perfectly dry Close the flask with the stopper which carries the funnel tube and the tube connecting with the absorption apparatus Weigh separately *F* and *G* and attach them to the apparatus If two soda lime tubes are employed, weigh them separately and refill the first when the second increases materially in weight Nearly fill the tube *B* with sulfuric acid (1+5) and place the soda lime tube *C* in position Aspirate air thru the Geissler bulbs at a rate of about two bubbles per second Open the stopper of the funnel and allow the acid to run slowly into the flask, taking care that the evolution of gas is so gradual as not materially to increase the current thru the Geissler bulbs After all the acid has been introduced, close the stopcock in *B*, continue the aspiration, and gradually heat the contents of the flask to boiling While the flask is being heated the aspirator tube may be removed, altho when using ground glass joints many analysts prefer to aspirate during the entire operation Continue the boiling for a few min after the water has begun to condense in *D*, then remove the flame, open the stopcock in tube *B*, and continue aspiration while the apparatus cools Remove bulbs *F* and *G* and weigh The increase in weight is due to CO_2

II Using Heidenhain's Apparatus[2]*—Official*

(Extremely accurate but too complicated for routine work)

REAGENTS

5 (a) *Calcium chloride*—Use $CaCl_2$ dehydrated at 200°, but not fused Grind coarsely and sift thru No 18 wire gauze to remove the extremely coarse, and thru No 30 wire gauze to remove the very fine particles

(b) *Soda lime*—Grind and sift the soda lime for the absorption tubes in the manner described under (a) The soda lime should not be too dry, as it must not absorb moisture to a greater degree than the $CaCl_2$

APPARATUS

6 Fill cylinder *A* with soda lime to remove CO_2 from the air passing thru the apparatus A thick layer of cotton at the upper end prevents soda lime dust from being carried over Connect the cylinder *A* by means of a perforated rubber stopper and a bent glass tube having a stopcock (*B*) and a capillary constriction (*C*) with a short piece of rubber tubing to which is attached a short piece of glass tubing (*E*), fitted with a perforated rubber stopper The stopper fits tightly into the constriction of *D* the funnel of which is cylindrical in shape, ¾ inch in diameter at the upper end ¾ inch at the lower end and 4 inches long The stem of *D* passes thru a doubly perforated rubber stopper and extends almost to the bottom of the evolution flask (*F*), which is ordinarily of 150 cc capacity but in the case of foaming liquids may hold 300 cc Thru the second perforation in the stopper connect flask *F* with a reflux condenser (*G*) To the upper end of the condenser attach a U tube containing a little $CaCl_2$ (to be renewed when it has liquefied) to retain the bulk of the moisture Connect this U tube with a second U tube (*A*) filled at *I* with a 3 inch column of pumice stone impregnated with $CuSO_4$ and completely dehydrated at 150°, the remainder of the tube being filled with fine $CaCl_2$ Connect the U-tube A with a bent glass tube having a

stopcock (*L*), which is closed when the apparatus is not in use Next attach the absorption U tubes (*M*) and (*N*), which are ¼ inch in diameter and 5 inches long the first filled mainly with soda lime but containing a little $CaCl_2$ at the end where the air current enters, the second filled one half with soda lime and one half with $CaCl_2$ the $CaCl_2$ being placed at the side where the air current leaves Connect *N* with a guard tube (*O*) filled with $CaCl_2$ on the side toward *N* and with soda lime on the side toward *P*, the latter being a small U tube trapped with glycerol to indicate the passage of the air current Connect *P* with a safety bottle (*R*) to receive any water which may be sucked back from the aspirator and connect *R* with a 4 liter aspirator bottle (*S*)

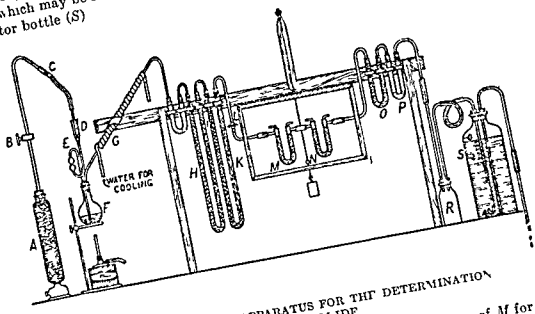

FIG 14 —HEIDENHAIN'S APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE

The tubes *M* and *N* should hold about 20 g making the capacity of *M* for CO_2 almost 1 g and that of *N* for moisture 0 2 g *M* should be refilled when its weight has increased 0 75 g and *N* after increase of 0 1 g in weight Use the best grade of rubber for all connections, applying a trace of castor oil as a lubricant For connections of the weighed tubes use rubber tubing boiled in weak lye washed, and dried Also apply a little castor oil wiping it off thoroly before connecting the tubing Before using the apparatus fill *H* and *K* with CO_2 in order to saturate the $CaCl_2$ with the gas and exhaust after several hours

DETERMINATION

7 To find the allowable rapidity of the air current used during the determination proceed as follows Charge the apparatus exactly as for an analysis leaving out the carbonate Begin to aspirate at the rate of about 50 cc per min After 2 liters has been aspirated weigh the tubes *M* and *N* If they have lost weight repeat the experiment with 40 cc per min and continue until the weight of the tubes remains constant If the work has been done cautiously the first tube will show a loss equal to the gain of the second tube Do not exceed the safe speed thus found

Shortly before weighing open *M* and *N* for a moment to allow equalization of air, then weigh at the air temp of the balance room and note the thermometer and barometer readings Connect the tubes with the apparatus and test the tightness of the joints by closing *A* at the bottom, opening all the cocks starting the aspirator,

and observing P, in which the liquid should soon come to a standstill Then disconnect the aspirator, close B, and remove F Put the sample into P, using about 1 g of Na_2CO_3 or $CaCO_3$ or about 2 g of baking powder, connect F, and start condenser G Introduce 50 cc of HCl (1+2 5) thru D, lifting E slightly and allowing only small quantities of the dilute acid to enter at a time Light the burner under F, heat to boiling, and then reduce the flame to keep the liquid just at the boiling point If no more air passes P, start the aspiration When the water stops running from S, open B carefully and adjust the outflow of the aspirator by raising or lowering the syphon to one half the safe speed

After M has cooled, increase the current to the full safe speed and aspirate 3 liters, continuing boiling to the end of the aspiration After the tubes have reached the temp of the balance room, open for a moment and weigh When extreme accuracy is desired, note again the thermometer and barometer readings and apply correction to the following formula

$$-(A^2-A^1)\times T \text{ and } +(B^2-B^1)\times B, \text{ in which}$$

A^1 = the temperature at first weighing in degrees C,
A^2 = the temperature at second weighing in degrees C,
B^1 = the barometric pressure at first weighing in mm, and
B = the barometric pressure at second weighing in mm

T and B are constants found from the following formulas

$$T = V\times 0\ 0000039 \text{ g},$$
$$B = V\times 0\ 0000015 \text{ g, in which}$$

0 0000039 = change in weight of 1 cc of air for 1°,
0 0000015 = change in weight of 1 cc of air for 1 mm pressure,

and the value of V is obtained from

$$V = \frac{G}{2\ 7}+\frac{F}{2\ 0}-\frac{G+F}{8\ 5},$$

representing the differential volume affected by temperature and pressure and being a constant for the tubes, and in which

G = the weight of the empty tubes,
F = the weight of the fillings
2 7 = the specific gravity of glass,
2 0 = the specific gravity of filling,
8 5 = the specific gravity of brass,
$\frac{G}{2\ 7}+\frac{F}{2\ 0}$ = volume of tubes and fillings, and
$\frac{G+F}{8\ 5}$ = volume of brass weights

Gasometric Method[3]*—Official*

8 REAGENTS

(a) *Sulfuric acid*—(1+5)

(b) *Displacement soln*—Dissolve 100 g of NaCl or $Na_2SO_4\ 10H_2O$ in 350 cc of H O Add approximately 1 g of $NaHCO_3$ and 2 cc of methyl orange indicator [VI, 3 (f)] and then sufficient H_2SO_4 (1+5) to make just acid (a decided pink color) Stir until all CO is removed This soln is used in the gas measuring tube and leveling bulb and seldom needs to be replaced

9 APPARATUS

Connect a decomposition flask (*A*) by means of a glass T tube (*B*), provided with a stopcock (*C*), to a graduated gas measuring tube (*D*), which in turn is connected with a leveling bulb (*E*) For *A* always use a 250 cc wide mouthed extraction flask of Pyrex or other resistant glass fitted with a two holed rubber stopper thru one hole of which passes the extended tip of a 25 cc buret (*F*) and thru the other a glass tube of the same diameter as the connecting T tube Use a buret graduated in cc at 20°, numbered at 5 cc intervals and provided with an extra long tip bent to pass thru the rubber stopper Connect the glass tube leading from the decomposition flask to the T tube by means of rubber tubing to permit rotation of the flask Use a gas measuring tube graduated in cc at 20 , the zero mark being placed at a point 25 cc below the top marking to allow for graduating upwards from 0 to 25 cc and downward from 0 to 200 cc By means of a long rubber tube connect the gas measuring tube with the leveling bulb, which has a capacity of about 300 cc

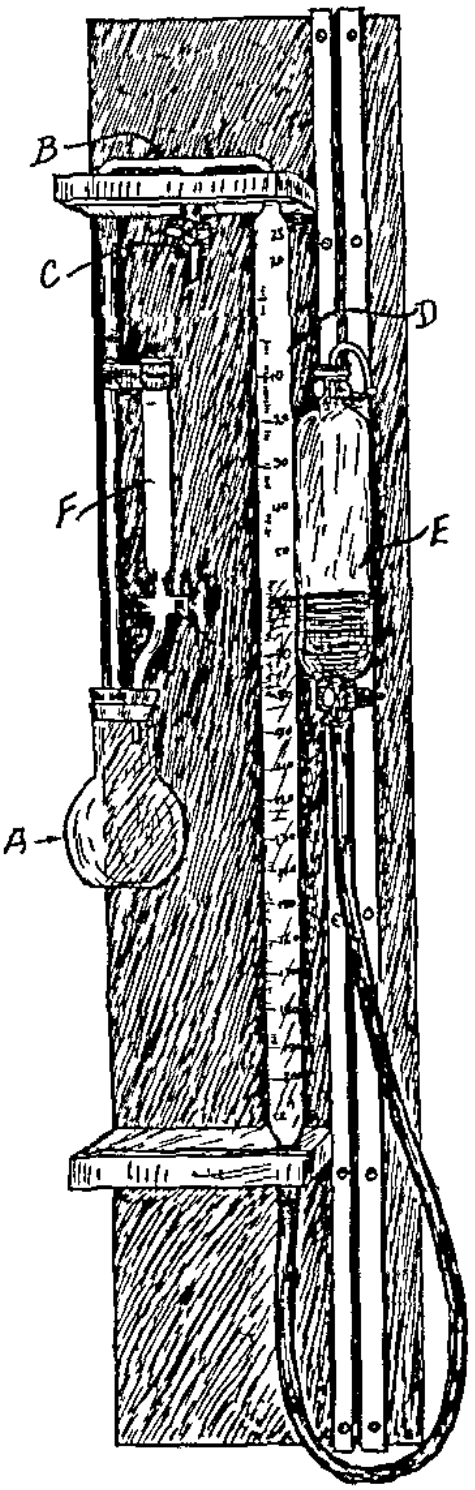

FIG 15—APPARATUS FOR THE GASOMETRIC DETERMINATION OF CARBON DIOXIDE

10 DETERMINATION

Weigh 1 7 g of the sample, prepared as directed under 1, into flask *A* and connect this flask with the apparatus (Fig 15) Open stopcock *C* and by means of the leveling bulb *E* bring the displacement soln to the 10 cc graduation above the zero mark (This 10 cc is practically equal in volume to the volume of acid to be used in the decomposition) Allow the apparatus to stand 1–2 min to insure that the temp and pressure within the apparatus are the same as those of the room Close the stopcock lower the leveling bulb somewhat to reduce the pressure within the apparatus, and slowly run into the decomposition flask from buret *F* 10 cc of the dilute H_2SO_4 To prevent the liberated CO_2 from escaping thru the acid buret into the air keep the displacement soln in the leveling bulb at all times during the decomposition at a lower level than that in the gas measuring tube Rotate and then vigorously agitate the decomposition flask to secure intimate mixture of the contents Allow to stand for 5 min to secure

equilibrium Equalize the pressure in the measuring tube by means of the leveling bulb and read the volume of gas in the tube Observe the temp of the air surrounding the apparatus and also the barometric pressure at the time and multiply the number of cc of gas evolved by the factor given in the table for this temp and pressure Divide the corrected reading by 10 to obtain the percentage by weight of CO_2 in the sample

RESIDUAL CARBON DIOXIDE[5]

11 *Gravimetric Method—Official*

Weigh 2 g of the sample, prepared as directed under 1, into a flask suitable for the subsequent determination of CO_2, add 20 cc of cold H_2O, and allow to stand 20 min Place the flask in a metal drying cell surrounded by boiling H_2O and heat, with occasional shaking, for 20 min To complete the reaction and drive off the last traces of gas from the semi solid mass, heat quickly to boiling and boil for 1 min Aspirate until the air in the flask is thoroly changed, and determine the residual CO_2 by absorption, as directed under 4 or 7

12 *Gasometric Method[6]—Official*

(See Table 24, correction factors for the gasometric determination of CO_2)

Place 1 7 g of the sample, prepared as directed under 1, in the decomposition flask, 9 (*A*), add 20 cc of H_2O and allow to stand 20 min Place the flask in a metal drying cell surrounded by boiling H_2O and heat, with occasional shaking, for 20 min To complete the reaction heat quickly to boiling and boil for 1 min Cool to room temp connect the flask to the apparatus described under 9, and determine the CO_2 present by treating with 10 cc of the dilute H_2SO_4 as directed under 10 To prevent foaming add 1–3 drops of caprylic alcohol to the baking powder in the decomposition flask

13 AVAILABLE CARBON DIOXIDE—OFFICIAL

Subtract the residual CO_2 from the total CO_2

NEUTRALIZING VALUE

14 *I Of Acid Reacting Materials Other Than Phosphates—Official*

Dissolve 1 g of the sample in hot H_2O and titrate with 0 2 *N* NaOH, using phenolphthalein indicator

15 *II Of Monocalcium Phosphate[7]—Tentative*

Weigh 0 84 g of monocalcium phosphate into a small beaker or casserole, add 25 cc of H_2O and 5 drops of a 0 2% soln of phenolphthalein[8], and titrate with 0 2 *N* NaOH, to a faint pink Heat to boiling, boil 1 min, and again continue the titration, while the soln is hot to a faint pink color, adding the bulk of the standard alkali soln rapidly and with vigorous stirring The total buret reading multiplied by 2 equals the neutralizing strength of 100 parts of phosphate in terms of $NaHCO_3$

16 TARTARIC ACID FREE OR COMBINED (QUALITATIVE TEST)[9]—TENTATIVE

(Applicable in the presence of phosphates)

Shake repeatedly about 5 g of the sample with about 250 cc of cold H_2O in a flask and allow the insoluble portion to subside Decant the soln thru a filter and evaporate the filtrate to dryness Powder the residue, add a few drops of 1% resorcin soln and about 3 cc of H_2SO_4 and heat slowly Tartaric acid is indicated by a *rose-red* color, which is discharged on dilution with water

17 TOTAL TARTARIC ACID—OFFICIAL

(Applicable only in the absence of Al salts, Ca salts, and phosphates)

Into a 500 cc porcelain casserole or similarly shaped dish weigh 1 88 g of the sample prepared as directed under 1 Add 10 cc of H_2O and then 10 cc of HCl (1+1) cautiously to avoid loss due to the sudden evolution of CO Heat gently until most of the starch present is hydrolized Add slowly and with constant stirring 15 cc of K_2CO_3 soln containing 328 g of K_2CO_3 per liter, boil gently on a hot plate for 1 min, and evaporate on a steam bath to incipient crystallization Remove from the steam bath and add dropwise with constant stirring, 3 cc of glacial acetic acid Add 2 cc more of the glacial acetic acid and continue the stirring for 3 min Add 150 cc of 95% alcohol carefully rinsing down the sides of the dish with the alcohol stirring vigorously for 5 min, and let stand for at least 1 hour Decant thru a Gooch crucible containing a thin layer of paper pulp or thru filter paper on a perforated disk, and wash largely by decantation, with 95% alcohol until the combined filtrate and washings measure 500 cc Test the last few cc of filtrate with dilute litmus tincture to be sure the precipitate has been properly washed Return the paper pulp, or disk, containing a part of the precipitate to the residue in the porcelain dish and add 120 cc of hot H_2O Add sufficient 0 2 *N* alkali soln to neutralize most, but not all, of the acidity Boil the soln for 5 min and complete the titration with 0 2 *N* alkali, using phenolphthalein indicator The number of cc of 0 2 *N* alkali times 2 equals the percentage of K bitartrate to which 0 15% is to be added to compensate for loss due to solubility 1 cc of 0 2 *N* alkali = 0 02641 g of tartaric anhydride 0 03001 g of tartaric acid, and 0 03763 g of K bitartrate

18 FREE TARTARIC ACID (QUALITATIVE TEST)—OFFICIAL

Extract 5 g of the sample with absolute alcohol and evaporate the alcohol from the extract Dissolve the residue in dilute NH_4OH transfer to a test tube add a good sized crystal of $AgNO_3$ and heat gently Tartaric acid is indicated by the formation of a silver mirror If desired the absolute alcoholic extract may be tested as directed under 16

STARCH

19 *I Direct Inversion Method—Official*

(For baking powders and baking chemicals free from calcium)

Weigh 5 g of the powder into a 500 cc volumetric flask and proceed as directed under XXVII, 23

20 *II Indirect Method*[12]*—Official*

(For baking powders and baking chemicals containing calcium)

Mix 5 g of the powder with 200 cc of HCl (1+11) in a 500 cc volumetric flask and allow the mixture to stand for an hour, shaking frequently Filter on an 11 cm hardened filter taking care to obtain a clear filtrate Rinse the flask once without attempting to remove all the starch, and wash the paper twice with cold H_2O Carefully wash the starch from the paper back into the flask with 200 cc of H_2O Add 20 cc of HCl (sp gr 1 125) and proceed as directed under XXVII, 23 The treatment with dilute hydrochloric acid (1+11) without dissolving the starch, removes effectively the Ca, which otherwise would be precipitated as tartrate by the alkaline Cu soln

21 *III Modified McGill Method—Tentative*

Digest 1 g of the powder with 150 cc of HCl (1+11) for 24 hours at room temp, with occasional shaking Filter on a Gooch crucible, wash thoroly with cold H_2O and then once with alcohol and once with ether Dry at 110° (4 hours is usually sufficient) cool, and weigh Burn off the starch, weigh again, and determine the starch by difference The results by this method on cream of tartar powders and tartaric acid powders agree closely with those obtained by Cu reduction The results on other types of baking powders are usually satisfactory, but in some instances they may be over 2% too high

ALUMINUM

I Qualitative Test—Official[a]

II Qualitative Test[11]*—Tentative*

(In presence of phosphates)

22 REAGENTS

(a) *Hydrochloric acid* —Approximately normal soln Dilute 9 cc of HCl to 100 cc

(b) *Ammonium acetate soln* —3 *N* Dissolve 23 1 g of NH_4-acetate in H_2O and dilute to 100 cc

(c) *Aurintricarboxylic acid* —0 1% soln Dissolve 1 g in H_2O and dilute to 100 cc

23 DETECTION

Dissolve 1–5 g of the baking powder in 5 cc of *N* HCl and 5 cc of 3 *N* NH_4 acetate Add 5 cc of a 0 1% soln of aurintricarboxylic acid, mix and allow the lake formation to take place Make the soln alkaline with NH_4OH containing a small quantity of $(NH_4)_2CO_3$ A bright persistent red precipitate indicates the presence of Al

III By precipitation with Phenylhydrazine[1]*—Tentative*

24 REAGENTS

(a) *Ammonium bisulfite soln* —Pass SO_2 into a cool soln of NH_4OH (1+1) until the color of the soln becomes distinctly yellow

(b) *Phenylhydrazine bisulfite soln* —To a few cc of phenylhydrazine add gradually a saturated soln of SO until the precipitate of phenylhydrazine sulfite, which at first separates out in crystals, is almost redissolved If the precipitate is completely dissolved add a drop or two of phenylhydrazine until a slight precipitate is obtained Filter the soln before using (From 5–10 cc of this soln in 100 cc of H_2O is sufficient strength for washing the $Al(OH)_3$ precipitate If well stoppered, this concentrated soln of phenylhydrazine bisulfite will keep indefinitely)

(c) *Dilute hydrochloric acid* —Add 10 volumes of H_2O to 4 of strong HCl

25 DETERMINATION

Ignite 3 g of baking powder at a temp not exceeding 550 As soon as the C has burned off take up the residue in HCl (4+10) and boil gently to assist soln Filter into a 300 cc volumetric flask and wash with hot H_2O Ignite the insoluble residue and filter paper in a Pt crucible and fuse the residue with about 2 g of Na_2CO_3 Dissolve the fused mass in water and HCl and transfer to the volumetric flask containing the original filtrate Cool, and make up to volume

Transfer 100 cc aliquots to 100 cc beakers Heat nearly to boiling, add dilute NH_4OH until a slight permanent precipitate forms, then just redissolve this pre-

[a] See Note 7 p xvii

cipitate with a drop or two of dilute HCl Add, dropwise with constant stirring, 10 or 12 drops of a saturated soln of NH_4HSO_3 Then add to the hot soln sufficient phenylhydrazine to precipitate the $Al(OH)_3$ completely (1 or 2 cc is generally enough an excess colors the soln yellow) If a permanent precipitate does not form at this point, add dilute NH_4OH carefully, dropwise, just to a permanent precipitate and then complete the precipitation by adding a few more drops of the phenyl hydrazine Let stand a few min for the precipitate to settle then filter while still warm Wash the precipitate with warm H O containing the phenylhydrazine bisulfite until the washings give no test for iron when yellow NH_4 sulfite is added

Place the filter paper containing the precipitate in a weighed Pt crucible Dry char, and ignite at a low temp After the filter paper has completely burned, continue the ignition at a bright red heat to constant weight Weigh quickly with the cover on the crucible as the precipitate is very hydroscopic A second weighing is always necessary The precipitate consists of Al O_3 and Al phosphate

Fuse the ignited precipitate with about 2 g of Na_2CO_3 and dissolve the fusion in HNO_3 (1+9) Transfer to a 250 cc beaker and boil to insure that all the phosphoric acid is in the ortho state Cool Transfer to a 200 cc flask, make up to volume and use 50 cc aliquots to determine the P_2O_5 Multiply the weight of P_2O_5 obtained by 4 and subtract the product from the weight of combined precipitates obtained above The difference is the weight of Al_2O_3 in 1 g of baking powder

Weight $Al_2O_3 \times 100$ = percentage of Al_2O_3

Percentage of $Al_2O_3 \times 4.749$ = percentage of $Na_2Al_2(SO_4)_4$

If the baking powder contains a significant quantity of SiO_2, remove it by evaporating the HCl soln of the powder to dryness and dehydrating at 105° for 2 hours Add to the dry mass 10 cc of HCl and 100 cc of H_2O boil filter off the SiO_2, and proceed as in 24

Ash[13]

26 INSOLUBLE ASH AND PREPARATION OF SOLUTION—OFFICIAL

Char 5 g of the sample in a Pt dish at a heat below redness Boil the carbonaceous mass with HCl (1+2 5) filter into a 500 cc volumetric flask, and wash with hot H_2O Return the residue together with the paper, to the Pt dish, and burn to a white ash Boil again with the dilute HCl filter wash, unite the two filtrates and dilute to 500 cc Incinerate the residue after the last filtration and weigh the ash insoluble in acid

27 IRON AND ALUMINUM—OFFICIAL

Draw a 100 cc aliquot of the soln prepared as directed under 26, and separate SiO_2 if necessary Mix the soln with 10% Na phosphate soln in excess Add strong NH_4OH until a permanent precipitate is obtained then HCl dropwise until the precipitate is dissolved Bring the soln to a boil and boil for 2–3 min mix with a considerable excess of 50% NH_4 acetate soln and 4 cc of 80% acetic acid As soon as the precipitate of Al phosphate mixed with Fe phosphate, has settled collect on a filter, wash with hot H_2O ignite and weigh Fuse the mixed phosphates with 10 parts of Na_2CO_3 dissolve in H_2SO_4 (1+6), reduce with zinc and determine the iron by titration with a standard permanganate soln (XXXVII, 58) In the same soln determine the phosphoric acid as directed under II, 7 or 10 To obtain the weight of Al_2O_3 subtract the sum of the weights of Fe_2O_3 and P_2O_5 from the weight of the mixed phosphates

28 CALCIUM—OFFICIAL

Heat the combined filtrate and washings obtained under 27 to 50° and add in

excess of saturated NH_4-oxalate soln Allow to stand in a warm place until the precipitate has settled, filter, wash the precipitate with hot H_2O, dry, and ignite over a Bunsen burner and finally over a blast lamp Cool in a desiccator and weigh as CaO

29 POTASSIUM AND SODIUM—OFFICIAL

Evaporate an aliquot of the soln, prepared as directed under 26, nearly to dryness to remove the excess of HCl, dilute, and heat to boiling While still boiling add 10% $BaCl_2$ soln as long as a precipitate forms and then enough saturated $Ba(OH)_2$ soln to make the liquid strongly alkaline As soon as the precipitate has settled, filter and wash with hot H_2O, heat the filtrate to boiling, add sufficient $(NH_4)_2CO_3$ [1 part of $(NH_4)_2CO_3$ in 5 of NH_4OH soln (1+12)] to precipitate all the Ba, filter, and wash with hot H_2O Evaporate the filtrate to dryness and ignite the residue below redness to remove NH_4 salts Add to the residue a little H_2O and a few drops of the $(NH_4)_2CO_3$ soln Filter into a weighed Pt dish, evaporate, ignite below redness, and weigh the mixed K- and Na chlorides Determine K in the mixed chlorides as directed under II, 46, beginning with "Digest the residue with hot H O, filter thru a small filter " Calculate the K so found to its equivalent of KCl and subtract this result from the weight of the mixed chlorides to obtain the weight of NaCl

30 PHOSPHORIC ACID—OFFICIAL

Mix 5 g of the sample with a little $Mg(NO_3)_2$ soln, dry, ignite, dissolve in HCl (1+2 5), and dilute the soln to a definite volume In an aliquot of the soln determine phosphoric acid as directed under II, 7 or 10

31 SULFURIC ACID[14]—OFFICIAL

Boil 5 g of the sample for 1 5 hours with a mixture of 300 cc of H_2O and 15 cc of HCl Filter, wash filter thoroly with hot H_2O cool the combined filtrate and washings, and dilute to a volume of 500 cc Determine H_2SO_4 in an aliquot of 100 cc as directed under XII, 25

32 AMMONIA—OFFICIAL

Introduce 2 g of the sample into a distillation flask, add 300–400 cc of H_2O and an excess of NaOH soln (1+1), connect with a condenser, and distil into a measured volume of standard acid Titrate the excess of acid in the distillate with standard alkali, using methyl red or cochineal indicator

LEAD

I Electrolytic Method[15]*—Official*

33 REAGENTS

(a) *Hydrochloric acid* —Dilute 1 volume of HCl with 2 volumes of H_2O
(b) *Nitric acid* —Dilute HNO_3 with an equal volume of H_2O
(c) *Acetic acid* —Dilute 1 volume of acetic acid with 4 volumes of H_2O
(d) *Potassium dichromate soln* —A saturated water soln of $K_2Cr_2O_7$

34 APPARATUS

(a) *Direct current* —The potential should be constant and not less than 6 volts A 3 cell, 6-volt storage battery will suffice
(b) *Voltmeter*
(c) *Ammeter* —Capacity 1 0 ampere and graduated to 0 01 ampere
(d) *Rheostat* —Not less than 100 ohms resistance

(c) *Glacial acetic acid* —Redistil glacial acetic acid and store in bottles made from Pb free glass

(d) *Alkaline ammonium acetate soln* —Mix 350 cc of the redistilled glacial acetic acid with 650 cc of H_2O, dilute 500 cc of strong NH_4OH with 500 cc of H_2O, and mix the two solns Store in bottles made from Pb free glass

(e) *Potassium chromate soln* —Dissolve 65 g of K_2CrO_4 in 100 cc of H_2O by heating gently Allow the soln to come to room temp and filter

37 DETERMINATION

Weigh 100 g of the sample, prepared as directed under 1, transfer to a 2 liter lipped beaker, and add in small portions at a time 750 cc of the dilute H_2SO_4 When frothing has ceased mark the volume of the mixture on the side of the beaker Heat on a hot plate to boiling and continue boiling for 3–4 min , then heat on a steam bath until the starch is hydrolized, which procedure requires 20–30 min The mixture will have a yellow color Remove from the steam bath and add with stirring $CaSO_4$ that has been finely powdered in a mortar and rubbed with H_2O to a thin paste It is not necessary to add $CaSO_4$ because monocalcium phosphate baking powder itself forms sufficient insoluble residue To combination baking powders containing in part monocalcium phosphate add 10 g of $CaSO_4$ and to other baking powders add 15 g of $CaSO_4$ Cool, dilute to the original volume with H_2O, add with stirring 1 liter of filtered 95% alcohol, cover beaker, and let stand overnight By means of a siphon that can be controlled by a pinch cock transfer the clear supernatant liquid to a Buchner funnel containing 3 layers of a very retentive, close textured filter paper, using suction To the moist residue in the beaker add 100 cc of the acid alcohol water mixture, stir well, let settle, and decant thru the same filter Repeat this operation, *using a fresh 100 cc portion of the acid alcohol-water mixture* Wash the beaker, the residue, and the filter with alcohol 70% by volume passing the washing thru the filter until the filtrate is nearly free from acid Discard the filtrate and washings and wash the filter flask Transfer the filter and contents to the original beaker and extract the $PbSO_4$ from the residue by treating with 100 cc portions of the alkaline NH_4-acetate soln heating to boiling, and filtering thru a new filter in an ordinary funnel Five extractions are necessary Transfer the filtrate which will measure about 500 cc, to a lipped beaker, neutralize with the redistilled glacial acetic acid, using litmus paper as indicator, and add 10 cc of the glacial acetic acid in excess Heat nearly to boiling add 25 cc of the K_2CrO_4 soln, cover, and let stand 48 hours at room temp , stirring occasionally Filter thru a weighed Gooch crucible wash well with cold H_2O, dry at 125° for at least 45 min , cool in a desiccator, and weigh The weight of $PbCrO_4$ multiplied by the factor 0 641 gives the weight of Pb

FLUORIDES[31]—TENTATIVE

38 REAGENTS

(a) *Sulfuric acid* —98 5% Add sufficient fuming H_2SO_4 to ordinary H_2SO_4 to give a soln containing about 99% of H_2SO_4 Heat in a large beaker, preferably on a hot plate for about 1 hour after it begins to fume strongly in order to remove free SO_2 and SO If the final product contains more than 98 5% of H_2SO_4 dilute with the correct quantity of H_2SO_4 or if it contains less than 98%, add more fuming acid In either case the acid as finally used should contain 98–98 5% of H_2SO_4 as determined by titration with standard alkali

(b) *Silver sulfate* —Dissolve 10 g of $(Ag)_2SO_4$ in 100 cc of 98 5% H_2SO_4 and heat until the soln fumes strongly in order to remove volatile impurities

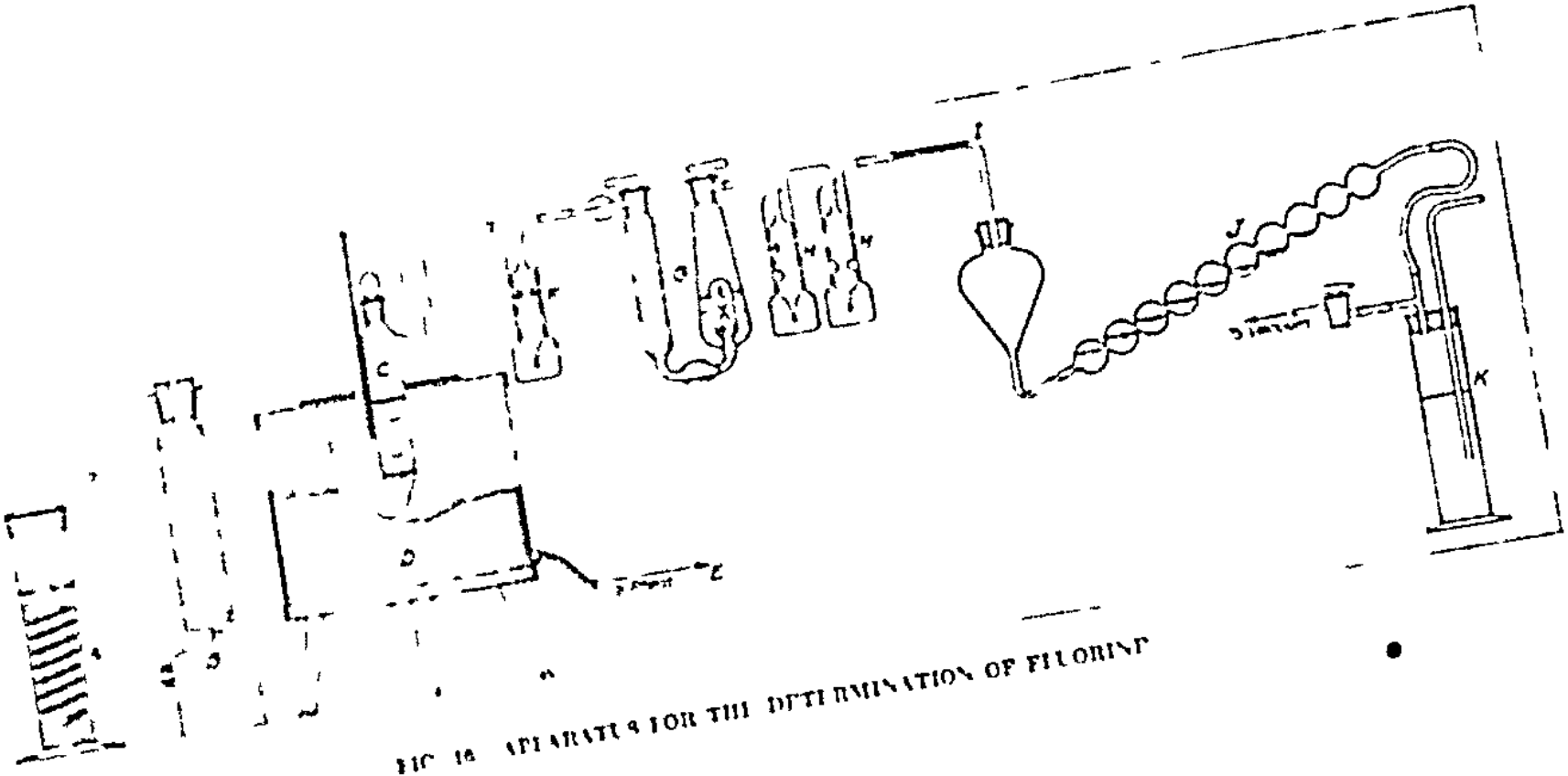

FIG. 18. APPARATUS FOR THE DETERMINATION OF FLUORINE

(c) *Chromium trioxide*—Dry at 105–110°, grind to a fine powder, and make a suspension containing a large excess of the solid CrO_3 in 98 5% H_2SO_4

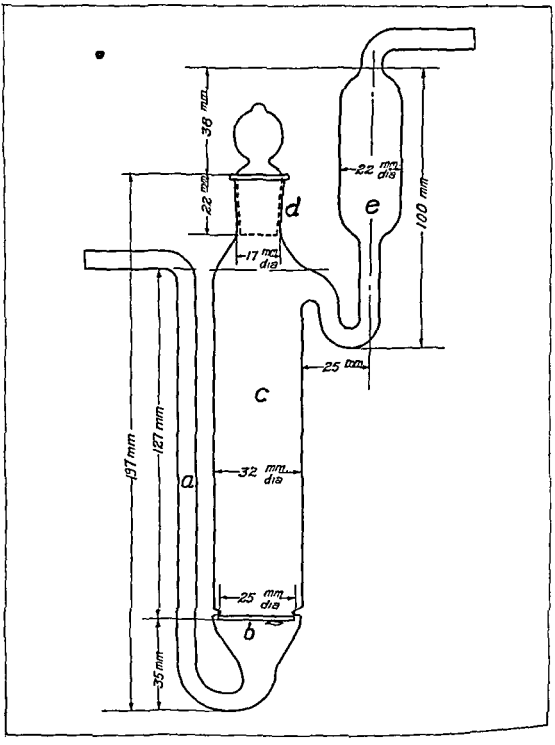

FIG 17—REACTION FLASK USED IN THE DETERMINATION OF FLUORINE

(d) *Silica*—Grind the commercial grade of quartz flour to 200 mesh and ignite at a low red heat to remove moisture and organic matter

(e) *Magnesium nitrate soln*—Dissolve 10 g of $Mg(NO_3)_2$ in H_2O and dilute to 100 cc

facilitate soln of the white scum that forms on the surface of the acid (The presence of Fl is characterized by the formation of this white scum) Digest for about 1 hour, then discontinue heating, and continue aeration for 15–20 min longer Disconnect the Meyer sulfur-tube, transfer its contents to a 500 cc flask and make up the final volume of soln and rinsings to 200–250 cc Place 10 cc of the standard HCl in an other flask of the same size and dilute to the same volume Heat both flasks to boiling and boil for 5 min Cool to about 50° and titrate the contents of each flask with 0 1 *N* NaOH, using phenolphthalein as indicator To correct for sulfate that may be present, add a small quantity of 10% $BaCl_2$ to the titrated soln after acidifying with HCl and compare the resulting turbidity of the soln with that produced on adding 0 05–0 3 cc of 0 1 *N* H_2SO_4 to equal volumes of solns containing the same quantities of $BaCl_2$ and HCl

From the total NaOH titration subtract the equivalents of the F blank on the reagents of the HCl used in the absorption tube, and of the sulfate found in the titrated soln to obtain the net NaOH equivalent to the F content of the sample 1 cc of 0 1 *N* NaOH soln = 0 0019 g of F

42 ARSENIC—TENTATIVE

Introduce 5 g of the sample directly into the generator described under XXIX, 2, add 10 cc of H_2O, a little at a time to prevent foaming over, and then 15 cc of As free HCl, introducing it drop by drop until foaming ceases Heat on a steam bath until a drop of the mixture, when diluted and treated with I soln, shows no blue color Then dilute to about 30 cc with H_2O and continue from this point as directed under XXIX, 4, beginning with "Add 5 cc of KI reagent " Make the blank and the standards for comparison by the use of the As free HCl of the same concentration as that used in the determination

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XVI

ANALYSIS OF THE ASH—OFFICIAL

17

Proceed as directed under XII and XXVI

PRESERVATIVES—OFFICIAL

18

Proceed as directed under XXXII

COLORING MATTERS—TENTATIVE

19

Proceed as directed under XXI

METALS—TENTATIVE

20

Proceed as directed under XXIX

ANTHRANILIC ACID ESTER

Colorimetric Method—*Official*

(Use when the sample contains less than 500 mg per liter.)

REAGENTS

21

(a) *Hydrochloric acid soln*.—Dilute 83 cc of HCl to 1 liter with H_2O.

(b) *Sodium nitrite soln*.—Dissolve 2 g of $NaNO_2$ in 100 cc of H_2O.

(c) *Hydrazine sulfate soln*.—Dissolve approximately 3 g of hydrazine sulfate in 100 c

100 cc of H_2O.

(d) *Sodium-α naphthol 2 sulfonate soln*.—Dissolve 5 g of the sulfonate in 100 c

of H_2O.

(e) *Sodium carbonate soln*.—Dissolve 25 g of Na_2CO_3 in 75 cc of H_2O.

(f) *Standard soln of methyl anthranilate*.—Dissolve 0.25 g of methyl anthranil

in 60 cc of 95% (by volume) alcohol and dilute with H_2O to 250 cc.

APPARATUS

22

(a) *Steam generator filled with H_2O*.—An oil can holding 1 gallon will serve the

purpose.

(b) *Distillation flask*.—A Kjeldahl flask of about 750 cc capacity, with shortened

neck, about 10 inches in height over all.

(c) *Spray tube*.—A glass tube with a small perforated bulb at the end. Passes

thru a rubber stopper and reaches to the bottom of the distillation flask.

(d) *Connecting bulb*.—A Kjeldahl bulb with bent connecting tube.

(e) *Worm condenser*.—Having a water jacket 10–12 inches long. The outlet tu

is extended to reach into the bottom of the receiving flask.

(f) *Receiving flask*.—A 500 cc Erlenmeyer flask.

DETERMINATION

23

Place enough H_2O in the receiving flask to just cover or seal the end of the

tended condenser tube. Place 10–100 cc of the sample of flavor in the distilla

flask; add, if necessary, sufficient H_2O to make the volume 100 cc, insert the stopper

carrying the spray tube and connecting bulb, and connect with the condenser and

receiving flask. Immerse the distillation flask in a water bath to the level of the con

tents and when the sample has attained the temp of the nearly boiling bath connect

with the steam generator and pass a rapid current of steam thru the sample until

about 300 cc of distillate has been collected.

Disconnect the apparatus and wash out the condenser with a little H_2O. Add to

the distillate 25 cc of the HCl soln and 2 cc of the $NaNO_2$ soln, mix well, and let

stand for exactly 2 min. Add 6 cc of the saturated soln of hydrazine sulfate and mix

well for a min., so that the liquid comes in contact with all parts of the flask that

may have been touched by the soln when it contained free nitrous acid Keep the liquid in the flask in rapid motion, add quickly 5 cc of the Na-α naphthol 2 sulfonate soln, and then add immediately 15 cc of the Na_2CO_3 soln Dilute the colored soln to 500 cc with H_2O, mix and compare the color of an aliquot with the color of a stand ard or set of standards prepared as nearly as possible at the same time Calculate and express results as mg of anthranilic acid ester, as methyl anthranilate per liter of sample

Gravimetric Method[2]—*Official*

(Use when the sample contains 500 mg or more per liter)

REAGENTS

24

(a) *Hydrochloric acid soln* —Dilute 83 cc of HCl to 1 liter with H_2O

(b) *Sodium nitrite soln* —Dissolve 2 g of $NaNO_2$ in 100 cc of H_2O

(c) *α naphthol soln* —Dissolve 0 2 g of α naphthol in 100 cc of 30% (by volume) alcohol

(d) *Sodium bicarbonate soln* —Dissolve 8 4 g of $NaHCO_3$ in 100 cc of H_2O

APPARATUS

25

The apparatus used is described under 22

DETERMINATION

26

Place in the distillation flask a quantity of the sample of flavor that contains from 50–125 mg of anthranilic acid ester and dilute if necessary, to 100 cc with H_2O Subject the sample to steam distillation as directed in 23, collecting about 400 cc of distillate Have the H_2O in the bath near the boiling point when the bath is placed under the distillation flask also have the H_2O in the steam generator boiling and make the connection immediately

Wash out the condenser with a little H_2O and dilute the distillate to 500 cc Mix and to a 200 cc aliquot add 5 cc of the HCl soln and 5 cc of the $NaNO_2$ soln Mix well and let stand for 1 min Mix 25 cc of the α naphthol soln and 6 cc of the $NaHCO_3$ soln pour the diazotized soln into the mixture and let stand for 10 min Fold two Whatman No 1 or S & S No 590 filter papers 12 5 cm in diameter, and determine the difference in their weights by placing one on each pan of the balance and counterpoising with added weights Place the heavier inside the lighter paper fit into a funnel and moisten Pour the mixture thru this filter and wash the pre cipitate 7 or 8 times using a total of about 100 cc of H_2O for this purpose Fill the filter only to within 1 cm of the top Place the funnel carrying the filter and washed precipitate in an oven and dry for about 10 min at a temp of 100° Then separate the filter papers and dry them for about 1 hour at the same temp Ascertain the dif ference in weights dry again weigh again and repeat this procedure until the differ ence in weights remains constant From this constant difference in weights subtract the original difference in weights of the 2 filter papers and multiply the result by 0 493, to obtain the weight of anthranilic acid ester as methyl anthranilate Calcu late and express as grams per liter of sample

SELECTED REFERENCES

[1] J Assoc Official Agr Chem , 11, 16 (1928)

[2] Ibid , 47

XVII BEERS, WINES AND DISTILLED LIQUORS

(Unless otherwise noted express results as grams per 100 cc)

BEERS

1 PREPARATION OF SAMPLE—OFFICIAL

Remove CO_2 by transferring the sample to a large flask and shaking vigorously or by pouring back and forth between beakers The temp of the beer should not fall below 20°

2 COLOR—TENTATIVE

Determine the depth of color of the sample in a $\frac{1}{2}$ inch cell with a Lovibond tintometer, using the beer scale Express the result in terms of a $\frac{1}{4}$ inch cell

3 SPECIFIC GRAVITY —OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer as directed under 24

4 ALCOHOL—OFFICIAL

Determine as directed under 25

EXTRACT

5 *Method I—Official*

Measure, at 20° 25 cc of the CO_2 free beer into a weighed, flat bottomed Pt dish approximately 85 mm in diameter, evaporate just to dryness on a steam bath, and heat to constant weight at 70° under a pressure of not to exceed 100 mm of Hg

6 *Method II—Tentative*

The immersion refractometer reading of the beer at 20° − the immersion refractometer reading of the distillate at 20° × 0 2571 = the grams of extract in 100 cc of beer

7 *Method III—Official*

Calculate the specific gravity of the dealcoholized beer by the following formula

$S = G + 1 - A$ in which

S = the sp gr of the dealcoholized beer,

G = the sp gr of the beer, and

A = the sp gr of the distillate obtained in the determination of alcohol 25

From Table 5 under XLII, ascertain the percentage by weight of extract in the dealcoholized beer corresponding to the value of S Multiply the figure thus obtained by S to obtain the grams of extract per 100 cc of beer

8 EXTRACT OF ORIGINAL WORT (APPROXIMATE)—OFFICIAL

Calculate the grams of extract per 100 cc in the original wort by the following formula

$O = 2A + E$ in which

O = extract of the original wort,

A = alcohol (g per 100 cc), and

E = extract of the dealcoholized beer (g per 100 cc)

See note 9 p xvii.

9 DEGREE OF FERMENTATION—OFFICIAL

Calculate the degree of fermentation by the following formula

$$D = \frac{100 \times 2A}{O}, \text{ in which}$$

D = degree of fermentation,
A = alcohol (g per 100 cc), and
O = extract of original wort

10 TOTAL ACIDS—OFFICIAL

Proceed as directed under 44 Express the result as lactic acid, g per 100 cc, 1 cc of 0 1 N NaOH = 0 0090 g of lactic acid

11 VOLATILE ACIDS—OFFICIAL

Proceed as directed under 46 Express the result as acetic acid, g per 100 cc

12 REDUCING SUGARS—OFFICIAL

Dilute 25 cc of the CO_2 free beer, measured at 20° with H_2O at the same temp to 100 cc Determine the reducing sugars in 25 cc of this soln as directed under XXXIV, 50 Express the result as grams of anhydrous maltose per 100 cc of beer

13 DEXTRIN—TENTATIVE

To 50 cc of the CO_2 free beer measured at 20° add 15 cc of HCl (sp gr 1 125) dilute to 200 cc, attach to a reflux condenser, and keep in a boiling water bath for 2 hours Cool nearly neutralize with NaOH soln complete to a volume of 250 cc, filter, and determine dextrose as directed under XXXIV, 59 or 61 From the number of g of dextrose per 100 cc of beer subtract 1 053 times the quantity of maltose, as found under 12, and multiply the remainder by 0 9 to obtain the number of g of dextrin per 100 cc of beer

14 DIRECT POLARIZATION—TENTATIVE

Read the polarization of the original sample in degrees Ventzke in a 200 mm tube at 20° If the beer is turbid clarify by shaking with alumina cream filter, and correct the reading for dilution

15 GLYCEROL—OFFICIAL

Proceed as directed under 27

16 ASH—OFFICIAL

Evaporate to dryness 25 cc of the CO_2 free sample, measured at 20°, and proceed as directed under XXVII, 8

17 PHOSPHORIC ACID—OFFICIAL

To 25 cc of the CO_2 free beer measured at 20°, add 20 cc of 2% Ca acetate soln evaporate to dryness, and ignite at low redness to a white ash Add 10–15 cc of boiling HNO_3 (1 + 9) and determine P_2O_5 as directed under II, 10

18 PROTEIN—OFFICIAL

Measure at 20° 25 cc of the CO_2-free beer into a Kjeldahl digestion flask, add a small quantity of tannin to prevent frothing, evaporate to dryness determine N as directed under II, 19, 22 or 24 multiply the result by 6 25 and calculate the percentage of protein

19 PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII

20 COLORING MATTERS—TENTATIVE

Proceed as directed under XXI

21 METALS—TENTATIVE

Proceed as directed under XXIX

WINES

22 PHYSICAL EXAMINATION—TENTATIVE

Note and record the following (1) Whether the container is "bottle full", (2) the appearance of the wine, whether it is bright or turbid and whether there is any sediment, (3) condition when opened, whether still, gaseous, or carbonated, (4) color and depth of color, (5) odor, whether vinous, acetous, pleasant, or foreign, and (6) taste whether vinous, acetous, sweet, dry, or foreign

23 PREPARATION OF SAMPLE—OFFICIAL

If gas is contained in the wine, remove it by pouring the sample back and forth in beakers

Filter the wine regardless of appearance, before analyzing and determine immediately the specific gravity and such ingredients as alcohol, acids, and sugars, which are liable to change thru exposure

24 SPECIFIC GRAVITY —OFFICIAL

Determine the specific gravity at 20/4° (in vacuo) by means of a *pycnometer* as follows Carefully clean the pycnometer by filling with a saturated soln of CrO_3 in H_2SO_4, allowing to stand for several hours emptying, and rinsing thoroly with H_2O Fill the pycnometer with recently boiled distilled H_2O previously cooled to 16–18°, place in a water bath cooled to the same temp and allow the bath to warm slowly to 20° Adjust the level of the H_2O to the proper point on the pycnometer, put the perforated cap or stopper in place, remove from the bath, wipe dry *with* a clean cloth, and after allowing to stand for 15–20 min , weigh Empty, rinse several *times* with alcohol and then with ether, remove the ether fumes, allow the instrument to become perfectly dry, and weigh Ascertain the weight of contained H_2O at 20° in air (W of the formula below) by subtracting the weight of the empty pycnometer from its weight when full Cool the sample to 16–18°, adjust the level of the liquid to the proper point on the pycnometer, put the perforated cap or *stopper* in place wipe dry and weigh as before Ascertain the weight of the contained *sample* at 20° in air (S of the formula below) by subtracting the weight of the empty pycnometer from its weight when filled with the sample Calculate the specific gravity in vacuo by the following formula

$$G = \frac{S + 0.00105W}{1.00282W}, \text{ in which}$$

G = corrected specific gravity of sample at 20/4° in vacuo,
W = weight of contained H_2O at 20° in air, and
S = weight of contained sample at 20° in air

25 ALCOHOL—OFFICIAL

(a) *By volume*.—Measure 100 cc of the liquid at 20° into a 300–500 cc distillation flask and add 50 cc of H_2O Attach the flask to a vertical condenser by means of a bent tube distil almost 100 cc, and make to a volume of 100 cc at 20° (Foaming

See note 9 p xvii.

ch sometimes occurs especially with young wines may be prevented by the addi of a small quantity of tannin) To determine the alcohol in wines that have dergone acetous fermentation and contain an abnormal quantity of acetic acid, xactly neutralize the portion taken with NaOH soln before distillation (This is ary, however, for wines of normal taste and odor) Determine the specific gravity of the distillate at 20°/4° as directed under 24 and obtain the corresponding centage of alcohol by volume from Tables 19-21, XLII

(b) *Grams per 100 cc* —From the specific gravity of the distillate, obtained under (a) ascertain the corresponding alcohol content in g per 100 cc from Tables 19-21, XLII

(c) *By weight* —Divide the number of g in the 100 cc of distillate as obtained in (b), by the weight of the sample as calculated from its specific gravity

(d) *By immersion refractometer* —Verify the percentages of alcohol as determined under (a) and (c) by ascertaining the immersion refractometer reading of the dis te and obtaining the corresponding percentages of alcohol from Tables 19-21, XLII

GLYCEROL IN DRY WINES

26 *Method I (By Direct Weighing)—Official*

Evaporate 100 cc of the wine in a porcelain dish on a water bath to a volume of about 10 cc Treat the residue with about 5 g of fine sand and 4–5 cc of milk of lime (containing 15 g of CaO per 100 cc) for each g of extract present and evapo rate almost to dryness Treat the moist residue with 50 cc of alcohol 90% by vol ume remove the substance adhering to the sides of the dish with a spatula and rub the whole mass to a paste Heat the mixture on a water bath, with constant stirring, to incipient boiling and decant the liquid thru a filter into a small flask Wash the residue repeatedly by decantation with 10 cc portions of hot 90% alcohol until the filtrate amounts to about 150 cc Evaporate the filtrate to a sirupy consistency in a porcelain dish on a hot but not boiling, water bath transfer the residue to a small glass stoppered, graduated cylinder with 20 cc of absolute alcohol and add 3 por tions of 10 cc each of anhydrous ether shaking thoroly after each addition Let stand until clear pour off thru a filter and wash the cylinder and filter with a mix ture of 2 parts of absolute alcohol to 3 parts of anhydrous ether also pouring the wash liquor thru the filter Evaporate the filtrate to a sirupy consistency, dry for an hour at the temp of boiling H_2O, weigh ignite, and weigh again The loss on ignition gives the weight of glycerol

27 *Method II (By Oxidation with Dichromate)—Official*

Evaporate 100 cc of the wine in a porcelain dish on a water bath, the temp of which is maintained at 80–90 to a volume of 10 cc Treat the residue with about 5 g of fine sand and 5 cc of milk of lime (containing 15 g of CaO per 100 cc) Proceed from this point as directed under XXXIII, 63, beginning with the clause evaporate almost to dryness with frequent stirring " except to dilute the soln of glycerol after treatment with $(Ag_2)CO_3$ and Pb acetate to a volume of 100 cc instead of 50 cc Observe the precautions given concerning the temp at which all evaporations are to be made

28 GLYCEROL IN SWEET WINES—OFFICIAL

With wines in which the extract exceeds 5 g per 100 cc heat 100 cc to boiling in a flask and treat with successive small portions of milk of lime until the wine becomes first darker and then lighter in color Cool add 200 cc of 95% alcohol allow the pre cipitate to subside, filter, and wash with 95% alcohol Treat the combined filtrate and washings as directed under 26 or 27

29 GLYCEROL-ALCOHOL RATIO—OFFICIAL

Express this ratio as Y 100, in which Y is obtained by multiplying the percentage weight of glycerol by 100 and dividing the result by the percentage of alcohol by weight

EXTRACT

30 *I From the Specific Gravity of the Dealcoholized Wine—Official*

Calculate the specific gravity of the dealcoholized wine by the following formula

$S = G + 1 - A$, in which

S = specific gravity of the dealcoholized wine,

G = specific gravity of the wine, 24, and

A = specific gravity of the distillate obtained in the determination of alcohol 25 (a)

From Table 5, under XLII, ascertain the percentage by weight of extract in the dealcoholized wine corresponding to the value of S Multiply the figure thus obtained by the value of S to obtain the g of extract per 100 cc of wine

31 *II By Evaporation—Official*

(a) *In dry wines, having an extract content of less than 3 grams per 100 cc*—Evaporate 50 cc of the sample on a water bath to a syrupy consistency in a 75 cc flat bottomed Pt dish, approximately 85 mm in diameter Heat the residue for 2–5 hours in a drying oven at the temp of boiling H_2O cool in a desiccator, and weigh as soon as the dish and contents reach room temp

(b) *In sweet wines*—If the extract content is between 3 and 6 g per 100 cc, treat 25 cc of the sample as directed under (a) If the extract exceeds 6 g per 100 cc, however, the result, obtained as directed under 30, is accepted, and no gravimetric determination is attempted because of the inaccurate results obtained by drying levulose at a high temp

32 NON SUGAR SOLIDS—OFFICIAL

Determine the non sugar solids (sugar-free extract) by subtracting the quantity of reducing sugars before inversion, 33, from the extract, 30 or 31 If sucrose is present in the wine, determine the non sugar solids by subtracting the sum of reducing sugars before inversion and the sucrose from the extract

33 REDUCING SUGARS—OFFICIAL

(a) *Dry wines*—Place 200 cc of the wine in a porcelain dish, exactly neutralize with normal NaOH, calculating the quantity required from the determination of acidity, 44, and evaporate to about ½ the original volume Transfer to a 200 cc flask add sufficient neutral Pb-acetate soln to clarify, dilute to the mark with H_2O, shake, and pass thru a folded filter Remove the Pb with dry K oxalate and determine reducing sugars as directed under XXXIV, 38

(b) *Sweet wines*—With sweet wines, approximate the sugar content by subtracting 2 from the result in the determination of the extract and employ such a quantity of the sample that the aliquot taken for the Cu reduction shall not exceed 210 mg of invert sugar Proceed as directed under (a) except to take this smaller quantity of the sample for the determination

SUCROSE

34 *I By Reducing Sugars Before and After Inversion—Official*

Proceed as directed under XXXIV, 28, using the method given under XXXIV, 38 for the determination of reducing sugars

35 *II By Polarization—Official*

Polarize before and after inversion in a 200 mm tube, as directed under XXXIV, 22 or 23, a portion of the filtrate obtained under 33 In calculating the percentage of sucrose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc to the normal weight for the instrument

36 COMMERCIAL GLUCOSE—OFFICIAL

Polarize a portion of the filtrate obtained under 33, after inversion in a 200 mm jacketed tube at 87°, as directed under XXXIV, 29 In calculating the percentage of glucose do not fail to take into consideration the relation of the weight of the sample contained in 100 cc to the normal weight for the instrument

37 ASH—OFFICIAL

Proceed as directed under XXVII, 8, using the residue from 50 cc of the wine

38 ASH EXTRACT RATIO—OFFICIAL

Express results as 1 : *N*, in which *N* is the quotient obtained by dividing the g of extract per 100 cc by the g of ash per 100 cc

39 ALKALINITY OF THE WATER SOLUBLE ASH—OFFICIAL

Extract the ash obtained as directed under 37 with successive small portions of hot H_2O until the filtrate amounts to about 60 cc and proceed as directed under XXXIV, 13 Express the alkalinity in terms of the number of cc of 0 1 *N* acid required to neutralize the water soluble ash from 100 cc of the wine

40 ALKALINITY OF THE WATER INSOLUBLE ASH—OFFICIAL

Ignite the filter and residue from 39 in the Pt dish in which the wine was ashed and proceed as directed under XXXIV, 14 Express the alkalinity in terms of the number of cc of 0 1 *N* acid required to neutralize the water insoluble ash from 100 cc of the wine

41 PHOSPHORIC ACID—OFFICIAL

Dissolve the ash obtained as directed under 37 in 50 cc of boiling HNO_3 (1+9), filter wash the filter, and determine P_2O_5 in the combined filtrate and washings as directed under II, 7 or 10 If the ash ignites without difficulty no free phosphoric acid need be suspected Should there be any free acid, the ash remains black even after repeated leaching In such cases add Ca acetate or a mixture containing 3 parts of Na_2CO_3 and 1 part of $NaNO_3$ to avoid loss of P_2O_5 before attempting to ash

42 SULFURIC ACID—OFFICIAL

Precipitate directly the H_2SO_4 in 50 cc of the wine by means of 10% BaI_2 soln after acidifying with a small excess of HCl and determine the resulting $BaSO_4$ as directed under XII, 25 Allow the precipitate to stand for at least 6 hours before filtering Report as SO_3 using the factor 0 3430

43 CHLORIDES—OFFICIAL

To 100 cc of dry wine or 50 cc of sweet wine, add sufficient Na_2CO_3 to make distinctly alkaline Evaporate to dryness, ignite at a heat not above low redness cool, extract the residue with hot H_2O acidify the water extract with HNO_3 (1+4) and determine chlorides as directed under XII, 33 or 35

XVII

TOTAL ACIDS—OFFICIAL

44

Measure 20 cc of the wine into a 250 cc beaker, heat rapidly to incipient boiling, and immediately titrate with 0 1 *N* NaOH soln Determine the end point with neutral 0 05% azolitmin soln as an outside indicator Place the indicator in the cavities of a spot plate and spot the wine into the azolitmin soln The end point is reached when the color of the indicator remains unchanged by the addition to the wine of a few drops of 0 1 *N* alkali

In the case of wines that are artificially colored and therefore cannot be titrated satisfactorily in the above manner, it will be found helpful to use phenolphthalein powder (one part of phenolphthalein mixed with 100 parts of dry, powdered K_2SO_4) as an indicator Place this indicator in the cavities of a spot plate and spot the wine into the powder The end of the titration is indicated when the powder acquires a pink tint

Express the result in terms of tartaric acid 1 cc of 0 1 *N* NaOH soln = 0 0075 g of tartaric acid

VOLATILE ACIDS

Method I—Official

45

Heat rapidly to incipient boiling 50 cc of the wine in a 500 cc distillation flask and pass steam thru until 15 cc of the distillate requires only 2 drops of 0 1 *N* NaOH soln for neutralization Boil the H_2O used to generate the steam several minutes before connecting the steam generator with the distillation flask in order to expel CO_2 Titrate rapidly with 0 1 *N* NaOH soln, using phenolphthalein indicator The color should remain about 10 seconds Express the result as acetic acid 1 cc of 0 1 *N* NaOH soln = 0 0060 g of acetic acid

Method II—Official

46

Introduce 10 cc of the wine, previously freed from CO_2, into the inner tube of a modified Sellier distillation apparatus (Fig 18), add a small piece of paraffin to prevent foaming, and adjust the tube and its contents in place within the larger flask,

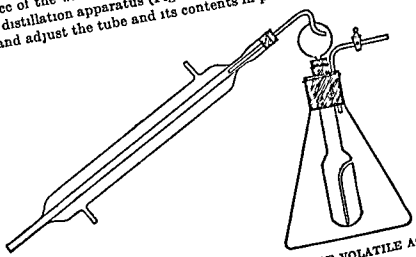

FIG 18—APPARATUS FOR THE DETERMINATION OF VOLATILE ACIDS

which contains 100 cc of recently boiled H_2O Connect with a condenser as illustrated in the figure and distil by heating the outer flask When 50 cc of the distillate has been collected empty the receiver into a beaker and titrate with 0 1 *N* NaOH soln, using phenolphthalein indicator Continue the distillation and titrate each

succeeding 10 cc of distillate until not more than 1 drop of standard alkali is required to reach the neutral point Usually 80 cc of distillate will contain all the volatile acids

47 FIXED ACIDS—OFFICIAL

To obtain the quantity of fixed acids, expressed as tartaric acid, multiply the quantity of volatile acids by 1 25 and subtract this product from the total acids

48 TOTAL TARTARIC ACID[1]—OFFICIAL

Neutralize 100 cc of the wine with N NaOH soln calculating from the acidity 44, the number of cc of N alkali necessary for the neutralization If the volume of the soln is increased more than 10% by the addition of the alkali, evaporate to approximately 100 cc Add to the neutralized soln 0 075 g of tartaric acid for each cc of N alkali added and after the tartaric acid has dissolved add 2 cc of glacial acetic acid and 15 g of KCl After the KCl has dissolved, add 15 cc of 95% alcohol, stir vigorously until the K bitartrate begins to precipitate and let stand in an ice box at 15–18 for at least 15 hours Decant the liquid from the separated K bitartrate on a Gooch crucible prepared with a very thin film of asbestos, or on filter paper in a Büchner funnel Wash the precipitate and filter 3 times with a few cc of a mixture of 15 g of KCl, 20 cc of 95% alcohol, and 100 cc of H_2O, using not more than 20 cc of the wash soln in all Transfer the asbestos or paper and precipitate to the beaker in which the precipitation was made wash the Gooch crucible or Büchner funnel with hot H_2O, using about 50 cc in all, heat to boiling, and titrate the hot soln with 0 1 N NaOH soln, using phenolphthalein indicator Increase the number of cc of 0 1 N alkali required by 1 5 cc to allow for the solubility of the precipitate 1 cc of 0 1 N alkali is equivalent, under these conditions to 0 015 g of tartaric acid To obtain the g of total tartaric acid per 100 cc of the wine, subtract the quantity of tartaric acid added from this result

49 FREE TARTARIC ACID AND CREAM OF TARTAR[2]—OFFICIAL

Calculate the free tartaric acid and cream of tartar in the following manner

Let A = total tartaric acid in 100 cc of wine, divided by 0 015

B = total alkalinity of the ash (sum of C and D),

C = alkalinity of water soluble ash and

D = alkalinity of water insoluble ash

Then

(1) If A is greater than B,
Cream of tartar = $0\ 0188 \times C$, and
Free tartaric acid = $0\ 015 \times (A - B)$,

(2) If A equals B or is smaller than B but greater than C
Cream of tartar = $0\ 0188 \times C$, and
Free tartaric acid = 0, and

(3) If A is smaller than C,
Cream of tartar = $0\ 0188 \times A$ and
Free tartaric acid = 0

TANNIN AND COLORING MATTER—OFFICIAL

50 REAGENTS

(a) *Oxalic acid* —0 1 N 1 cc = 0 00416 g of tannin

(b) *Standard potassium permanganate soln* —Dissolve 1 333 g of $KMnO_4$ in 1 liter of H_2O and standardize the soln against (a)

(c) *Indigo soln* —Dissolve 6 g of Na sulfindigotate in 500 cc of H_2O by heating cool add 50 cc of H_2SO_4, make up to 1 liter, and filter

(d) *Purified boneblack* —Boil 100 g of finely powdered boneblack with successive portions of HCl (1+3), filter, and wash with boiling H_2O until free from chlorides Keep covered with H_2O

51 DETERMINATION[4]

Dealcoholize 100 cc of the wine by evaporation and dilute with H_2O to the original volume Transfer 10 cc to a 2 liter porcelain dish and add about 1 liter of H_2O and exactly 20 cc of the indigo soln Add the standard $KMnO_4$ soln, 1 cc at a time, until the blue color changes to green, then add a few drops at a time until the color becomes golden yellow Designate the number of cc of $KMnO_4$ soln used as "a "

Treat 10 cc of the dealcoholized wine, prepared as above, for 15 min with bone black, filter, and wash thoroly with H_2O Add 1 liter of H_2O and 20 cc of the indigo soln and titrate with $KMnO_4$, as above Designate the number of cc of $KMnO_4$ used as "b

Then $a-b=c$, the number of cc of the $KMnO_4$ soln required for the oxidation of the tannin and coloring matter in 10 cc of the wine

52 CRUDE PROTEIN—OFFICIAL

Determine N in 50 cc of the wine as directed under II, 19, 22 or 24, and multiply the result by 6 25

53 PENTOSANS—OFFICIAL

Proceed as directed under XXVII, 29, except to use 100 cc of the wine and 43 cc of HCl in beginning the distillation Owing to the interference of sugars this determination can be made in dry wines only

54 GUM AND DEXTRIN—TENTATIVE

Evaporate 100 cc of the wine to about 10 cc and add 10 cc of 95% alcohol If gum or dextrin is present (indicated by the formation of a voluminous precipitate), continue the addition of alcohol, slowly and with stirring, until 100 cc has been added Let stand overnight, filter, and wash with alcohol, 80% by volume Dissolve the precipitate on the paper with hot H_2O, hydrolyze the filtrate and washings with HCl, and proceed as directed under XXVII, 23

55 NITRATES—TENTATIVE

(a) *White wine* —Treat a few drops of the wine in a porcelain dish with 2–3 cc of H_2SO_4 that contains about 0 1 g of diphenylamine[5] per 100 cc The deep blue color formed in the presence of nitrates appears so quickly that it is not obscured, even in sweet wine, by the blackening produced by the action of H_2SO_4 on the sugar

(b) *Red wine* —Clarify with basic lead acetate, filter, remove the excess of Pb from the filtrate with Na_2SO_4 filter again, and treat a few drops of this filtrate as directed under (a)

56 COLORING MATTERS—TENTATIVE

Proceed as directed under XXI

57 PRESERVATIVES—OFFICIAL

Proceed as directed under XXXII

The detection of added boric acid is somewhat difficult because a small quantity of it is normally present in certain wines Therefore a quantitative determination

l be made The determination of SO_2 must also be quantitative A small
, of salicylic acid is also normal in wine, and for that reason not more than
0 cc of the sample should be used in testing for that preservative

DISTILLED LIQUORS

58 SPECIFIC GRAVITY —OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, as directed under 24, or by means of a small, accurately graduated hydrometer

59 ALCOHOL BY WEIGHT—OFFICIAL

Weigh 20-25 g of the sample into a distillation flask, dilute with 100 cc of H_2O, and distil nearly 100 cc Weigh the distillate or make to volume at 20° In either case determine the specific gravity as directed under 58 Obtain the corresponding per centage of alcohol by weight from Tables 19-21, XLII, multiply this figure by the weight of the distillate, and divide by the weight of the sample taken to obtain the percentage of alcohol by weight

The alcohol content of the distillate may be checked by determining the immer sion refractometer reading and obtaining the percentage of alcohol from Table 22 under XLII

ALCOHOL BY VOLUME

60 *Method I—Official*

From the specific gravity of the distillate obtained under 59 ascertain the corre sponding percentage of alcohol by volume from Tables 19 21, XLII Multiply this figure by the volume of distillate and divide by the volume of the sample (calculated from the specific gravity) to obtain the percentage of alcohol by volume in the orig- inal sample

61 *Method II—Official*

Measure 25 cc of the sample at 20 into a distillation flask, dilute with 100 cc of H_2O distil nearly 100 cc make to volume at 20°, and determine the specific gravity as directed under 24 Obtain, from Tables 19-21, XLII, the corresponding per centage of alcohol by volume in the distillate and multiply by 4 to obtain the per centage of alcohol by volume in the original substance

The alcohol content of the distillate may be checked by determining the immer sion refractometer reading and obtaining the percentage of alcohol from Tables 19- 21, XLII

62 EXTRACT—OFFICIAL

Weigh, or measure at 20°, 100 cc of the sample, evaporate nearly to dryness on a steam bath, transfer to a water oven and dry at the temp of boiling H_2O for 2 5 hours

63 ASH—OFFICIAL

Proceed as directed under XXVII, 8, using the residue from the determination of the extract, 62

64 ACIDITY—OFFICIAL

Titrate 100 cc of the sample (or 50 cc diluted to 100 cc if the sample is dark) with 0 1 N alkali using phenolphthalein indicator Express the result as acetic acid 1 cc of 0 1 N alkali = 0 0060 g of acetic acid

See p 19 p xvii

METHYL ALCOHOL

73 *Trillat Method*[7]*—Official*

To 50 cc of the sample add 50 cc of H O and 8 g of lime and fractionate by the aid of Glinsky bulb tubes Dilute the first 15 cc of the distillate to 150 cc, mix with 15 g of $K_2Cr_2O_7$ and 70 cc of H_2SO_4 (1+5), and allow to stand for 1 hour, shaking occasionally

Distil, reject the first 25 cc, and collect 100 cc Mix 50 cc of the distillate with 1 cc of redistilled dimethylaniline, transfer to a stout tightly stoppered flask, and keep on a bath at 70–80 for 3 hours, shaking occasionally Make distinctly alkaline with NaOH soln and distil off the excess of dimethylaniline, stopping the distillation when 25 cc has passed over

Acidify the residue in the flask with acetic acid, shake, and test a few cc by adding 4 or 5 drops of a 1% suspension of PbO_2 If methyl alcohol is present, there occurs a blue coloration, which is increased by boiling Ethyl alcohol thus treated yields a blue coloration which changes immediately to green, later to yellow, and becomes colorless when boiled

74 *Riche and Bardy Method*[8]*—Official*

The following method depends on the formation of methylaniline violet

Place 10 cc of the sample previously redistilled over K_2CO_3 if necessary in a small flask with 15 g of I and 2 g of red P Keep in ice H_2O for 10–15 min or until action has ceased Distil on a water bath into about 30 cc of H_2O, the methyl and ethyl iodides formed Wash with dilute alkali to eliminate free I Separate the heavy, oily liquid that settles and transfer to a flask containing 5 cc of aniline If the action is too violent, place the flask in cold H_2O, if too slow, stimulate by gently warming the flask After an hour boil the product with H_2O, cool and add about 20 cc of 15% NaOH soln, when the bases rise to the top as an oily layer, fill the flask up to the neck with H_2O and draw them off with a pipet Oxidize 1 cc of the oily liquid by adding 10 g of a mixture of 100 parts of clean sand, 2 of NaCl, and 3 of $Cu(NO_3)_2$, mix thoroly, transfer to a glass tube, and heat to 90° for 8–10 hours Exhaust the product with warm alcohol, filter, and dilute to 100 cc with alcohol If the sample is free from methyl alcohol, the liquid has a red tint, but in the presence of 1% of methyl alcohol it has a distinct violet shade, with 2 5% the shade is very distinct and still more so with 5% To detect more minute quantities of methyl alcohol, dilute 5 cc of the colored liquid to 100 cc with H_2O and dilute 5 cc of this again to 400 cc *Heat the liquid thus obtained in a porcelain dish and immerse in it a fragment* of white merino (free from S) for 30 min If the alcohol is pure the w ol will remain white but if methyl alcohol is present the fiber will become violet, th depth of tint giving a fairly approximate indication of the proportion of methyl alcohol

75 *Immersion Refractometer Method*[9]*—Official*

Determine by the immersion refractometer at 20 the refraction of the distillate obtained in the determination of alcohol If, on reference to the table under 76, the refraction shows the percentage of alcohol agreeing with that obtained from the specific gravity, it may be assumed that no methyl alcohol is present If, however, there is an appreciable quantity of methyl alcohol, the low refractometer reading will at once indicate the fact *If the absence from the soln of refractive substances other* than H_2O and the alcohols is assured, this difference in refraction is conclusive of the presence of methyl alcohol

The addition of methyl alcohol to ethyl alcohol decreases the refraction in direct proportion to the quantity present hence the quantitative calculation is made read

ily by interpolation in the table under 76 of the figures for pure ethyl and methyl alcohol of the same alcoholic strength as the sample being used

Example—The distillate has a specific gravity of 0 97080, corresponding to 18 38% alcohol by weight and has a refraction of 35 8 at 20° by the immersion refractometer, by interpolation in the refractometer table the readings of ethyl and methyl alcohol corresponding to 18 38% alcohol are 47 3 and 25 4, respectively the difference being 21 9, 47 3 − 35 8 = 11 5, (11 5 − 21 9)100 = 52 5, showing that 52 5% of the total alcohol present is methyl alcohol

76

Scale readings on Zeiss immersion refractometer at 20° corresponding to each per cent by weight of methyl and ethyl alcohols

PER CENT ALCOHOL BY WEIGHT	SCALE READINGS		PER CENT ALCOHOL BY WEIGHT	SCALE READINGS		PER CENT ALCOHOL BY WEIGHT	SCALE READINGS		PER CENT ALCOHOL BY WEIGHT	SCALE READINGS	
	Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol
0	14 5	14 5	25	29 7	60 1	50	39 8	90 3	75	29 7	101 0
1	14 8	16 0	26	30 3	61 9	51	39 7	91 1	76	29 0	101 0
2	15 4	17 6	27	30 9	63 7	52	39 6	91 8	77	28 3	100 9
3	16 0	19 1	28	31 6	65 5	53	39 6	92 4	78	27 6	100 9
4	16 6	20 7	29	32 2	67 2	54	39 5	93 0	79	26 8	100 8
5	17 2	22 3	30	32 8	69 0	55	39 4	93 6	80	26 0	100 7
6	17 8	24 1	31	33 5	70 4	56	39 2	94 1	81	25 1	100 6
7	18 4	25 9	32	34 1	71 7	57	39 0	94 7	82	24 3	100 5
8	19 0	27 8	33	34 7	73 1	58	38 6	95 2	83	23 6	100 4
9	19 6	29 6	34	35 2	74 4	59	38 3	95 7	84	22 8	100 3
10	20 2	31 4	35	35 8	75 8	60	37 9	96 2	85	21 8	100 1
11	20 8	33 2	36	36 3	76 9	61	37 5	96 7	86	20 8	99 8
12	21 4	35 0	37	36 8	78 0	62	37 0	97 1	87	19 7	99 5
13	22 0	36 9	38	37 3	79 1	63	36 5	97 5	88	18 6	99 2
14	22 6	38 7	39	37 7	80 2	64	36 0	98 0	89	17 3	98 9
15	23 2	40 5	40	38 1	81 3	65	35 5	98 3	90	16 1	98 6
16	23 9	42 5	41	38 4	82 3	66	35 0	98 7	91	14 9	98 3
17	24 5	44 5	42	38 8	83 3	67	34 5	99 1	92	13 7	97 8
18	25 2	46 5	43	39 2	84 2	68	34 0	99 4	93	12 4	97 2
19	25 8	48 5	44	39 3	85 2	69	33 5	99 7	94	11 0	96 4
20	26 5	50 5	45	39 4	86 2	70	33 0	100 0	95	9 6	95 7
21	27 1	52 4	46	39 5	87 0	71	32 3	100 2	96	8 2	94 9
22	27 8	54 3	47	39 6	87 8	72	31 7	100 4	97	6 7	94 0
23	28 4	56 3	48	39 7	88 7	73	31 1	100 6	98	5 1	93 0
24	29 1	58 2	49	39 8	89 5	74	30 4	100 8	99	3 5	92 0
									100	2 0	91 0

77

COLORING MATTERS—TENTATIVE

Proceed as directed under XXI

78

WATER INSOLUBLE COLOR—TENTATIVE

Evaporate 50 cc of the sample just to dryness on a steam bath Take up with approximately 15 cc of cold H_2O filter and wash until the filtrate amounts to nearly 25 cc To this filtrate add 25 cc of absolute alcohol, or 26 3 cc of 95% alcohol and

make up to 50 cc with H_2O Mix thoroly and compare in a colorimeter with the original material Calculate from these readings the percentage of color insoluble in H_2O

79 COLORS INSOLUBLE IN AMYL ALCOHOL—TENTATIVE

Evaporate 50 cc of the sample just to dryness on a steam bath Dissolve the residue in H_2O and 95% alcohol and make to a volume of 50 cc, using a total volume of 26 3 cc of 95% alcohol Place 25 cc of this soln in a separatory funnel and add 20 cc of freshly shaken Marsh reagent (100 cc of pure amyl alcohol, 3 cc of syrupy H_3PO_4 and 3 cc of H_2O), shaking lightly so as not to form an emulsion Allow the layers to separate and repeat this shaking and standing twice After the layers have separated completely draw off the lower or aqueous layer, which contains the caramel, into a 25 cc cylinder and make up to volume with alcohol, 50% by volume Compare this soln in a colorimeter with the untreated 25 cc Calculate from this reading the percentage of color insoluble in amyl alcohol

80 CARAMEL[11]—TENTATIVE

Add 10 cc of paraldehyde to 5 cc of the sample in a test tube and shake Add absolute alcohol a few drops at a time, shaking after each addition until the mixture becomes clear Allow to stand Turbidity after 10 min is an indication of caramel

MARSH TEST FOR ARTIFICIAL COLORS—TENTATIVE

(Caramel and Some Coal Tar Dyes)

81 REAGENT

Marsh Reagent[11]—Prepare as directed under 79

82 DETERMINATION

Place 10 cc of the sample in a 20 cc test tube, add sufficient Marsh reagent to nearly fill the tube, and shake several times Allow the layers to separate, if the lower layer is colored it is an indication that the sample has been colored with caramel or a coal tar dye

In the absence of any color test 10 cc of the sample in the same manner, using sufficient fusel oil, amyl alcohol or pentasol to nearly fill the tube, and shake several times A deeply colored lower layer is an indication of a coal tar dye, its identity should be confirmed by using the methods under XXI

DETECTION OF METHANOL IN ALCOHOLIC BEVERAGES[12]

83 REAGENTS

(a) *Potassium permanganate soln*—Dissolve 3 g of $KMnO_4$ in 15 cc of 85% H_3PO_4 and make up to 100 cc with H_2O

(b) *Oxalic acid soln*—Dissolve 5 g of oxalic acid in 100 cc of H_2SO_4 (1+1)

(c) *Schiff's reagent*—Dissolve 0 2 g of Kahlbaum rosaniline hydrochloride in 120 cc of hot H_2O cool add 2 g of anhydrous Na_2SO_3 dissolved in 20 cc of H_2O, and 2 cc of HCl make up to 200 cc and store in well filled glass stoppered amber bottles

84 DETERMINATION

Dilute the alcoholic beverage to 5% total alcohol by volume Transfer 5 cc of this soln to a 6-inch test tube add 2 cc of the $KMnO_4$ soln, and let stand 10 min Remove the excess of $KMnO_4$ by the addition of 2 cc of the oxalic acid soln As soon as the $KMnO_4$ is decolorized add 5 cc of Schiff s reagent Mix thoroly and let stand 10 min If HCHO is present, the characteristic reddish purple color is produced

Run blanks on pure ethyl alcohol and on ethyl alcohol containing about 1% of methanol

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[6] Vasey, Guide to the Analysis of Potable Spirits, 1904, p 31
[7] Abs Analyst, 24, 13 211 212 (1899)
[8] Allen, Commercial Organic Analysis 4th ed , 1, 98 (1909)
[9] J Am Chem Soc 27, 961 (1905)
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XVIII COFFEE AND TEA

GREEN COFFEE

1 MACROSCOPIC EXAMINATION—TENTATIVE

A macroscopic examination usually shows the presence of excessive quantities of black and blighted coffee beans, coffee hulls, stones, and other foreign matter Separate these by hand picking and determine the quantity gravimetrically

2 COLORING MATTERS—TENTATIVE

Shake vigorously 100 g or more of the sample with cold H_2O or alcohol, 70% by volume Strain thru a coarse sieve and allow to settle Identify soluble colors in the soln and insoluble pigments in the sediment as directed under XXI

ROASTED COFFEE

3 MACROSCOPIC EXAMINATION—TENTATIVE

Artificial coffee beans are apparent from their regularity of form, and roasted legumes and lumps of chicory in whole roasted coffee can be picked out and identified microscopically For ground coffee sprinkle some of the sample on cold H_2O and stir lightly Fragments of pure coffee, if not overroasted, will float, while fragments of chicory legumes cereals, etc , will sink immediately, chicory coloring the H O a decided brown In all cases identify the particles that sink by microscopical examination

4 PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a sieve having holes 0 5 mm in diameter and preserve in a tightly stoppered bottle

5 MOISTURE—TENTATIVE

Dry 5 g of the sample at the temp of boiling H_2O under a pressure not to exceed 100 mm of Hg or at a temp of 105–110 under atmospheric pressure for 5 hours and subsequent periods of 1 hour each until constant weight is obtained For whole coffee, grind rapidly to a coarse powder and without sifting and unnecessary exposure to the air weigh portions for the determination For ground coffee, sample directly without further grinding

6 SOLUBLE SOLIDS—TENTATIVE

Place 4 g of the sample, prepared as directed under 4, in a 200 cc flask, add H_2O to the mark, allow the mass to infuse for 8 hours, with occasional shaking, let stand 16 hours longer without shaking, filter, evaporate 50 cc of the filtrate to dryness in a flat-bottomed dish dry at 100°, cool, and weigh

7 ASH—OFFICIAL

Proceed as directed under XXVII, 8, using the sample prepared as directed under 4

8 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 12, using the ash obtained under 7

ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

9 Proceed as directed under XXXIV, 13, using the filtrate obtained under 8

ASH INSOLUBLE IN ACID—OFFICIAL

10 Proceed as directed under XXXIII, 5, using the ash obtained as directed under 7 or the water insoluble ash obtained as directed under 8

SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

11 Proceed as directed under II, 7 or 10, using the soln obtained under 9

INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

12 Boil the insoluble ash obtained as directed under 8 with 25 cc of HCl (1+2), filter, wash thoroly with hot H_2O, and determine P_2O_5 in combined filtrate and washings as directed under II, 7 or 10

CHLORIDES—OFFICIAL

13 Proceed as directed under XII, 33 or 35,

CAFFEINE

Power Chesnut Method[1]*—Official*

14 Moisten 10 g of the sample, prepared as directed under 4, with 95% alcohol, transfer to a Soxhlet or similar extraction apparatus, and extract with 95% alcohol for 8 hours, exercising care to assure complete extraction Transfer the extract with the aid of hot H_2O to a porcelain dish containing 10 g of heavy MgO in suspension in 100 cc of H_2O (This reagent should meet the U S P requirements) Evaporate slowly on a steam bath with frequent stirring to a dry powdery mass Rub the residue with a pestle into a paste with boiling H_2O and transfer with hot H_2O to a smooth filter, cleaning the dish with a rubber tipped glass rod Collect the filtrate in a liter flask marked at 200 cc and wash with boiling H_2O until the filtrate reaches the mark Add 20 cc of H_2SO_4 (1+9) and boil gently for 30 min with a funnel in the neck of the flask Cool filter thru a moistened double paper into a separatory funnel and wash with small portions of H_2SO_4 (1+199) Extract with 6 successive 25 cc portions of $CHCl_3$ Wash the combined $CHCl_3$ extracts in a separatory funnel with 5 cc of 10% KOH soln Filter the $CHCl_3$ into an Erlenmeyer flask Wash the KOH soln with 2 portions of $CHCl_3$ of 10 cc each adding them to the flask, together with the $CHCl_3$ washings of the filter paper Evaporate or distil on a steam bath to a small volume (10 15 cc) transfer with $CHCl_3$ to a weighed beaker evaporate carefully dry for 20 min at 100° and weigh Test the purity of the residue by determining N and multiplying by the factor 3 464

Fendler Stuber Method (Modified)[2]*—Tentative*

15 (Adapted for quick results)

Treat a 10 g sample prepared as directed under 4, with 10 cc of NH_4OH (1+2) and 200 g of $CHCl_3$ in a glass stoppered bottle shake continuously by machine or hand for 20 min and chill in an ice bath Pour the entire contents of the bottle on a 21 cm fluted filter covering immediately with a watch glass Collect the filtrate with the funnel resting directly in the neck of a flask (previously weighed with stopper) and having the flask surrounded with ice Stopper as soon as the soln ceases to run from the funnel in a continuous stream and weigh Evaporate on a steam bath removing the last $CHCl_3$ with a current of air Digest the residue with 50 cc of hot H_2O for 10 min on a steam bath shaking frequently and let cool Treat the soln

with 20 cc (for roasted) and 10 cc (for unroasted) of 1% $KMnO_4$ soln and let stand 15 min at room temp , shaking occasionally Add 2 cc of H O_2 soln (100 cc of 3% H_2O_2, free of acetanilid, plus 1 cc of glacial acetic acid) If the liquid is still red or reddish, add the H_2O_2 soln 1 cc at a time until the excess of $KMnO_4$ is destroyed Place the flask on a steam bath for 15 min and add 0 5 cc portions of the H_2O_2 soln until the liquid ceases to become lighter Cool, and filter by suction thru a Gooch crucible washing with cold H O Transfer the filtrate to a separatory funnel and extract 6 times with 25 cc portions of $CHCl_3$ Evaporate the combined $CHCl_3$ extracts to a small volume transfer to a weighed beaker, finish evaporation, dry at 100° to constant weight (30 min is usually sufficient), and weigh the residue as caffeine The weight of the caffeine, multiplied by 2000 and divided by the weight of the $CHCl_3$ aliquot obtained from the first filtration, equals the percentage of caffeine in the 10 g sample Test the purity of the residue by determining N and multiplying by the factor 3 464

16 CRUDE FIBER—OFFICIAL

Proceed as directed under XXVII, 19, using the sample prepared as directed under 4

17 STARCH—TENTATIVE

Extract 5 g of the sample, prepared as directed under 4, on a hardened filter with 5 successive 10 cc portions of ether, wash with small portions of 95% alcohol until a total of 200 cc has passed thru, place the residue in a beaker, and proceed as directed under XXVII, 25

18 SUGARS[2]—TENTATIVE

Weigh 10 g of the sample, prepared as directed under 4, into a 250 cc volumetric flask, add 1 g of powdered NH_4NaHPO_4, and proceed as directed under XXVII, 20–22 Determine the reduced Cu in the Cu_2O precipitate either volumetrically as directed under XXXIV, 41, or electrolytically, as directed under XXXIV, 43

19 PETROLEUM ETHER EXTRACT—OFFICIAL

Dry 2 g of the sample, prepared as directed under 4, at 100°, extract with petroleum ether (b p 35–50) for 16 hours, evaporate the solvent, dry the residue at 100°, cool, and weigh

20 TOTAL ACIDITY—TENTATIVE

Treat 10 g of the sample, prepared as directed under 4, with 75 cc of alcohol 80% by volume, in an Erlenmeyer flask, stopper, and allow to stand 16 hours, shaking occasionally Filter, and transfer an aliquot of the filtrate (25 cc for green coffee, 10 cc for roasted coffee) to a beaker, dilute to about 100 cc with H_2O, and titrate with 0 1 *N* alkali using phenolphthalein indicator Express the result as the number of cc of 0 1 *N* alkali required to neutralize the acidity of 100 g of the sample

21 VOLATILE ACIDITY—TENTATIVE

Into a volatile acid apparatus (XVII, 44) introduce a few glass beads and over these place 20 g of the unground sample Add 100 cc of recently boiled H O place a sufficient quantity of recently boiled H_2O in the outer flask, and distil until the distillate is no longer acid to litmus paper (Usually 100 cc of distillate will be collected) Titrate the distillate with 0 1 *N* alkali using phenolphthalein indicator Express the result as the number of cc of 0 1 *N* alkali required to neutralize the acidity of 100 g of the sample

COATING AND GLAZING SUBSTANCES

22 SUGAR AND DEXTRIN—TENTATIVE

Introduce 100 g of the whole coffee into a beaker add exactly 300 cc of H_2O stir, and allow to stand 5 min stirring frequently Filter thru a dry filter and add care fully to the filtrate sufficient dry Pb acetate to precipitate all the caffe tannic acid avoiding an excess of the reagent Filter thru a dry filter and remove the Pb from the filtrate by the addition of a slight excess of dry powdered K-oxalate Filter thru a dry filter and determine reducing sugars as invert sugar in 50 cc of the filtrate, as directed under XXXIV, 38 Invert a 75 cc aliquot of the filtrate as directed under XXXIV, 23 (b) Cool, nearly neutralize with NaOH soln (1+1) dilute to 100 cc, and determine reducing sugars as invert sugar in the resulting soln as directed under XXXIV, 33 Measure a 100 cc aliquot of the filtrate into a 200 cc flask, add 10 cc of HCl (sp gr 1 12) and hydrolyze as directed under XXVII, 23 Cool, neutralize with NaOH soln (1+1), dilute to volume filter thru a dry filter and determine re ducing sugars as invert sugar in 50 cc of the filtrate as directed under XXXIV, 38 Calculate the reducing sugars in each instance to percentage by weight of the ori ginal coffee Calculate sucrose from the reducing sugars before and after inversion as directed under XXXIV, 28, and calculate dextrin as follows Subtract the reducing sugars after inversion from the reducing sugars after hydrolysis and multiply the difference by the factor 0 8605 to convert the result to dextrin

In some instances the presence of sucrose in the wa er extract may be verified by polarization The presence of dextrin in the water extract may be verified by polari zation as directed under XXXIV, 30, and by the erythro dextrin test (XXXIV, 95) performed on the water extract previous to clarification with Pb acetate

23 EGG ALBUMIN AND GELATIN—TENTATIVE

Treat 100 g of the whole coffee with 500 cc of H_2O and allow to stand for 5 min , stirring frequently Filter and treat separate portions of the filtrate with (1) a 5% soln of tannic acid, or (2) Millon s reagent (XX, 15) Boil a third portion of the fil trate In the presence of egg albumin a more or less heavy precipitate will be formed in each case As a confirmatory test treat an aliquot of the filtrate with an excess of tannic acid soln add a little salt if neces ary to secure flocculation of the precipi tate filter, and without washing introduce the paper and its contents into a Kjel dahl flask and determine N By this method coffee not coated with albumin or gelatin will yield less than 10 mg of N per 100 g of sample

24 CHICORY INFUSION—TENTATIVE

Cover 100-150 g of the whole coffee with H_2O, allow to soak 2 3 min stirring frequently and drain the aqueous washings thru a coarse sieve Wash the coffee upon the sieve with about 100 cc of H_2O and centrifugal e the combined washings Decant the clear liquid from the sediment, which should then be drained almost dry on filter paper Mount the sediment in chloral hydrate soln [XXXIII, 20 (g)] and examine under the microscope for elements of chicory

25 FATS AND WAXES—TENTATIVE

Treat 100 200 g of the beans with low boiling petroleum ether for 10 min pour off the petroleum ether and repeat the proce s Filter the combined extracts evapo rate and determine the index of refraction and the saponification number of the re idue as directed under XXXI, 8 and 22

26

TEA

DUST STEMS AND FOREIGN LEAVES—TENTATIVE

Place 1 g of the tea in a 300 cc casserole, add 200 cc of boiling H_2O, and allow to stand 15 min This treatment will cause the leaves to unroll and they will then be in condition for examination as to form and structure [5] A macroscopic examination will reveal the presence or absence of dust or stems Only those stems that remain floating after the leaf is thoroly infused should be regarded as woody stems [6] ("floaters")

27

PREPARATION OF SAMPLE—OFFICIAL

Grind the sample to pass thru a sieve having circular openings 0 5 mm in diameter

28

MOISTURE—OFFICIAL

Proceed as directed under XXVII, 2

29

WATER EXTRACT [7]—OFFICIAL

To 2 g of the ground sample in a 500 cc volumetric flask add 200 cc of hot H_2O and boil over a low flame for 1 hour, rotating occasionally Close the flask with a rubber stopper thru which passes a glass tube 30 inches long for a condenser Boil very slowly so that no steam escapes from the top of the air condenser Cool, dilute to volume mix thoroly, and filter thru a dry filter paper Transfer an aliquot of 50 cc to a weighed dish and evaporate to dryness on a steam bath Place in the oven, heat at 100° for 1 hour cool, and weigh

30

ASH—OFFICIAL

Proceed as directed under XXVII, 8

31

SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 12, using the ash obtained under 30

32

ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using the filtrate obtained under 31

33

ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using the insoluble ash obtained under 31

34

ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under XXXIII, 5, using the total ash obtained as directed under 30, or the insoluble residue obtained under 31

35

SOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under II, 7 or 10, using the soln of soluble ash obtained under 32

36

INSOLUBLE PHOSPHORIC ACID IN THE ASH—OFFICIAL

Proceed as directed under II, 7 or 10, using the soln obtained under 33

37

PETROLEUM ETHER EXTRACT—OFFICIAL

Proceed as directed under 19

38

PROTEIN—TENTATIVE

Determine N as directed under II, 19, 22 or 24 To obtain the percentage of N present as protein subtract the percentage of N present as caffeine from the percentage of total N Multiply this result by 6 25 to obtain the percentage of protein

39 CRUDE FIBER—OFFICIAL

Proceed as directed under XXVII, 19

40 VOLATILE OIL—TENTATIVE

Add 100 g of tea to 800 cc of H_2O, distil, extract the distillate several times with petroleum ether transfer the combined petroleum ether extracts to a weighed dish, evaporate at room temp, dry in a desiccator and weigh

CAFFEINE

41 *Power Chesnut Method*[8]*—Official*

Proceed as directed under 14

42 *Bailey Andrew Method*[9]*—Official*

To 5 g of the sample, prepared as directed under 27, in a 500 cc volumetric flask add 10 g of heavy MgO and 200 cc of H O Boil gently over a low flame for 2 hours, using a small bore glass tube 30 inches long as a condenser Cool, dilute to volume, and filter thru a dry paper Transfer an aliquot portion of 300 cc equivalent to 3 g of original material, to an Erlenmeyer flask of 1 liter capacity, add 10 cc of H_2SO_4 (1+9) and boil until the volume is reduced to about 100 cc Filter into a separatory funnel, washing the flask with small portions of H_2SO_4 (1+99), and shake 6 times with $CHCl_3$, using 25 20, 15 10 10 cc portions Treat the combined extracts with 5 cc of a 1% soln of KOH and when the liquids have completely separated draw off the $CHCl_3$ layer into a suitable flask or beaker Wash the alkaline soln in the separatory funnel with 2 portions of $CHCl_3$ of 10 cc each and unite the washings with the main bulk of extract Evaporate or distil off the $CHCl_3$ to a small bulk, transfer to a weighed flask, evaporate to dryness, and further dry in an oven at 100° to constant weight Test the purity of the residue by determining N and multiplying by the factor 3 464 This gives a value for anhydrous caffeine

TANNIN[10]—TENTATIVE

43 REAGENTS

(a) *Potassium permanganate soln* — Prepare a soln containing 1 33 g per liter and obtain its equivalent in terms of 0 1 *N* oxalic acid

(b) 0 1 *N oxalic acid*

(c) *Indigotine soln* —Prepare a soln containing 6 g of indigotine (free from indigo blue) and 50 cc of H_2SO_4 per liter

(d) *Gelatin soln* —Soak 25 g of gelatin for 1 hour in saturated NaCl soln, heat until the gelatin is dissolved cool, and dilute to 1 liter

(e) *Acid sodium chloride soln* — Acidify 975 cc of saturated NaCl soln with 25cc of H_2SO_4

(f) *Powdered kaolin*

44 DETERMINATION

Boil 5 g of the tea for 30 min with 400 cc of H O, cool, transfer to a 500 cc volumetric flask, and dilute to the mark To 10 cc of the infusion (filtered, if not clear) add 25 cc of the indigo carmine soln and about 750 cc of H_2O. Add the $KMnO_4$ soln from a buret, a little at a time while stirring until the color becomes light green then dropwise until the color changes to bright yellow or to a faint pink at the rim Designate the number of cc of $KMnO_4$ used as "*a*"

Mix 100 cc of the clear infusion of tea with 50 cc of the gelatin soln 100 cc of the acid NaCl soln and 10 g of the powdered kaolin and shake several min in a

stoppered flask After allowing the mixture to settle, decant thru a filter Mix 25 cc of the filtrate with 25 cc of the indigotine soln and about 750 cc of H_2O and ti trate with $KMnO_4$ as before The number of cc of $KMnO_4$ used subtracted from that obtained above, "*a*," gives the quantity of $KMnO_4$ required to oxidize the tannin 1 cc of 0 1 *N* oxalic acid =approximately 0 0012 g of tannin (gallotannic acid)

FACING

45 GENERAL—TENTATIVE

(1) Examine the ash obtained as directed under 30 for mineral pigments (cf XXI, 1), (2) shake a quantity of the tea with a large volume of water and remove the leaves by means of a sieve Allow the insoluble matter in the water portion to settle, filter and examine the residue on the filter paper for insoluble pigments as directed under XXI, 1 Catechu and other soluble substances, if used, will be found in the filtrate

46 PARAFFIN AND WAXY SUBSTANCES—TENTATIVE

Spread a quantity of the tea between two sheets of unglazed white paper and place thereon a hot iron Any greasy substance will stain the paper [11]

47 PIGMENTS USED FOR COLORING OR FACING[12]—TENTATIVE

Place 60 g of the tea in a 60 mesh, 5 to 6 inch sieve, provided with a top Sift a small quantity (approximately 0 1 g) of the dust upon a piece of semi glazed, white paper about 8 by 10 inches (To obtain the requisite quantity of dust, it is sometimes necessary to rub the leaf gently against the bottom of the sieve) Place the paper on a plain, firm surface, preferably glass or marble, and crush the dust by pressing firmly upon it a flat steel spatula about 5 inches long Repeat the crushing process until the tea dust is ground almost to a powder, when particles of coloring matter, if present, become visible as streaks on the paper Brush off the loose dust and ex amine the paper by means of a simple lens magnifying 7 5 diameters Bright light is essential to distinguish these particles and streaks In many cases the character of the pigment is indicated by the behavior of these streaks when treated with re agents and examined under a microscope The crushed particles of leaf of either black or green tea appear in such quantity that there is no chance of mistaking them for coloring or facing material Repeat this test, using black semi glazed paper for facings such as talc, gypsum, $BaSO_4$, or clay

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XIX CACAO BEAN AND ITS PRODUCTS

1 PREPARATION OF SAMPLE—OFFICIAL

Mix powdered products thoroly and preserve in tightly stoppered bottles. Chill sweet or bitter chocolate until it becomes hard and reduce to a finely granular condition by grating or shaving. Mix thoroly and preserve in a tightly stoppered bottle in a cool place.

MOISTURE

2 *Method I—Official**

Proceed as directed under XXVII, 2.

3 *Method II—Tentative*

Dry 2 g of the sample prepared as directed under 1, in a Pt dish in an air oven at 100° to constant weight. An Al dish may be used when the ash is not determined in the same sample. Report the loss in weight as moisture.

4 ASH—OFFICIAL

Proceed as directed under XXVII, 8, using sufficient sample to contain approximately 1 g of water, sugar and fat free material.

5 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 12, using the ash obtained under 4.

6 ALKALINITY OF THE SOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 13, using the filtrate from 5.

7 ALKALINITY OF THE INSOLUBLE ASH—OFFICIAL

Proceed as directed under XXXIV, 14, using the insoluble ash obtained under 5.

8 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as directed under XXXIII, 5, using the total ash as obtained under 4, or the water insoluble residue as obtained under 5.

9 TOTAL NITROGEN—OFFICIAL

Proceed as directed under II, 19, 22 or 24.

10 CASEIN IN MILK CHOCOLATE—TENTATIVE

It is unnecessary to defat the chocolate. Weigh 10 g of the chocolate prepared as directed under 1, into a 500 cc Erlenmeyer flask and add 250 cc of 1% Na_2-oxalate soln. Heat to boiling and boil gently for a few min., then cool, add 5 g of MgO, and filter. Determine N in 50 cc of this filtrate. Pipet 100 cc of the filtrate into a 200 cc volumetric flask and dilute almost to the mark with H_2O. Precipitate the casein by the addition of 2 cc of glacial acetic acid or 1 cc of H_2SO_4. Make to volume, shake, filter and determine N in 100 cc of the filtrate. The difference between the two N determinations gives the N derived from the casein which, multiplied by 6.38, gives the quantity of casein present in 2 g of the sample.

* See p. xxi.

18 EXAMINATION OF CACAO BUTTER

Saponify 5 g of the sample with 15 cc of the alcoholic KOH soln and evaporate the alcohol on a steam bath Run a blank on pure cacao butter at the same time Add 5 cc of H_2O and again evaporate to remove the last trace of alcohol Dissolve the soap in 100 cc of H_2O, cool to room temp , and add, while stirring, 100 cc of the saturated salt soln Allow to stand for 15 min , stirring several times during this period, and then separate the soap by filtration, using a Büchner funnel To 100 cc of the filtrate add, while stirring, 100 cc of the saturated salt soln and allow to stand for 15 min Only a slight precipitate should appear Filter, add to the filtrate a drop of phenolphthalein indicator, neutralize with HCl (1+3), and then add 0 5 cc of this reagent in excess If the sample consists of pure cacao butter, the soln when acidified will remain clear If coconut or palm kernel oil is present, the soln will become turbid or milky

19 EXAMINATION OF FAT EXTRACTED FROM MILK CHOCOLATE

Milk fat if present in cacao butter subjected to this test, produces a turbidity less in intensity than that produced by the same percentage of coconut or palm kernel oil For example cacao butter containing 10%, 15%, or 20% milk fat produces, respectively, no opalescence, a faint opalescence, or an opalescence For this reason, when the fat to be examined has been extracted from a cacao product that contains lactose or casein, multiply the percentage of lactose in the cacao product by 0 8, or the percentage of casein by 1 1, to obtain the percentage of milk fat in the product, and from this result calculate the percentage of milk fat in the total fat If this percentage corresponds to 15% or less, a blank of cacao butter containing 15% milk fat may be used, otherwise make up a mixture of cacao butter and milk fat in the proportions indicated by the calculations

Test the fat extracted from the sample under examination as directed under 18, but use the prepared mixture of cacao butter and milk fat instead of the pure cacao butter for the blank If the fat being tested contains coconut oil or palm kernel oil the last filtrate, when acidified, will be more turbid or milky than the blank

CRITICAL TEMPERATURE OF DISSOLUTION IN ACETIC ACID TEST—TENTATIVE

20 REAGENTS

(a) *Glacial acetic acid* —99 5%

(b) *Potassium hydroxide soln* —0 1 *N*

21 APPARATUS

Insert a thermometer reading to 0 1° into a cork that fits a 6x¾ inch test tube The thermometer should extend far enough into the tube so that the bulb will be covered by 10 cc of liquid Place the test tube in a larger tube (4 x 1½ inch) containing glycerol and hold firmly in place with a cork having a groove cut in the side to equalize the pressure when heat is applied

22 DETERMINATION

To remove traces of moisture, filter a portion of the sample to be examined thru a dry paper in an oven in which a temp of about 110° is maintained Allow the filtered sample to cool until barely warm and weigh 5 g of the sample and 5 g of the acetic acid reagent into the test tube Insert the cork holding the thermometer

and place the test tube in the glycerol bath Heat, and shake the apparatus frequently until a clear soln of the fat and acetic acid is obtained Allow the soln to cool with constant shaking without removing from the glycerol bath Note the temp at which the first sign of turbidity appears Make a similar test with the same acetic acid on a sample of pure cacao butter

Free fatty acids lower the turbidity temp A correction, therefore, must be made for the acid value of the sample If the strength of the acetic acid reagent is such that the turbidity temp of the pure cacao butter is approximately 90°, one unit of acid value will cause a reduction of 1 4° in the critical temp of dissolution If the turbidity temp is approximately 100°, one unit of acid value will cause a reduction of 1 2° For intermediate temp the reduction is proportional

Determine the acid value (mg of KOH required to neutralize the free fatty acids in 1 g of the sample) of both the sample and the pure cacao butter as directed under XXXI, 32, using 5 g of fat Multiply the acid value by the correction factor and add the result to the observed turbidity temp The figure obtained is the true critical temp of dissolution If the true critical temp of dissolution of the sample is lower than that of the pure cacao butter by more than 3° in the case of fat from chocolate liquors or sweet chocolates, and by more than 6 in the case of fat from milk chocolates adulteration with coconut, palm kernel, corn, peanut, cottonseed oils, etc or their stearines is indicated

ACETONE CARBON TETRACHLORIDE TEST—TENTATIVE

REAGENT

23 *Acetone carbon tetrachloride* —Mix equal volumes of acetone and CCl_4

DETERMINATION

24 Dissolve 5 cc of the warm fat, which has been previously filtered thru a dry filter paper in an oven at about 110 to remove traces of moisture in 5 cc of the acetone-CCl_4 reagent in a test tube Allow the soln to stand in ice H_2O for 20–30 min Run a blank on a sample of pure cacao butter at the same time If hydrogenated oil, tallow, oleostearin, or paraffin is present a white flocculent precipitate will soon appear If the H_2O is cold enough, cacao butter may solidify If a precipitate is formed, remove the sample from the ice H_2O and allow to remain at room temp for a time Solidified cacao butter will soon melt and go into soln but if the precipitate is due to any of the above mentioned possible adulterants a much longer time will be required

MELTING POINT—OFFICIAL

25 Proceed as directed under XXXI, 13 Melting point determinations upon cacao fat do not become normal until the fat has been kept for at least 24 hours in a cool place

INDEX OF REFRACTION—OFFICIAL

26 Proceed as directed under XXXI, 8

IODINE ABSORPTION NUMBER—OFFICIAL

27 Proceed as directed under XXXI, 18 or 20

SAPONIFICATION NUMBER—OFFICIAL

28 Proceed as directed under XXXI, 22

29 REICHERT MEISSL AND POLENSKE VALUES—*OFFICIAL*

Proceed as directed under XXXI, 26

CACAO SHELL

30 REAGENTS

(a) *Chloral hydrate soln* —Prepare as directed under XXXIII, 20(g)

(b) *Acidified chloral hydrate-glycerol soln* —Prepare as directed under XXXIII, 20(h)

31 APPARATUS

(a) *Centrifuge or suction filter*

(b) *Ruled slides* —Prepare in the laboratory as follows Warm an ordinary microscopic slide, dip in hot melted beeswax, drain, and cool Rule with a fine needle and etch with HF So place the rulings that at any point on the slide two lines are visible in the microscopic field

(c) *Cover-glasses* —No 2, 22 mm square

(d) *Compound microscope* —Use a magnification of approximately 120 mm (A higher magnification is helpful in the case of certain fragments difficult to identify)

32 PREPARATION OF STANDARDS

Prepare standard samples from clean cacao nibs and shells Grind and thoroly defat both nibs and shells separately until each passes thru a 100-mesh sieve Weigh, and mix the nib powder and shell powder in desired proportions, finally sieving each standard thru the 100 mesh sieve to insure thoro and uniform mixing

33 PREPARATION OF SAMPLE

(a) *Bitter chocolate and cocoa* —Completely remove the fat from a portion of the sample, prepared as directed under 1, by extracting 3 or 4 times with gasoline or ether in a centrifuge or on a suction filter If necessary, remove the sample to a mortar, grind and continue the defatting process to completion Dry, powder, and mix the sample thoroly by passing twice thru a 100-mesh sieve Weigh 2 mg on a tared ruled slide, or weigh and transfer to a ruled slide and mount with just sufficient chloral hydrate soln or acidified chloral hydrate glycerol soln to fill in under the cover glass Before applying the cover glass stir and spread the material with the point of a needle to secure a uniform mount Warm slightly for 15–20 min (do not boil) and let stand until the tissues have cleared (preferably about 12 hours for the chloral hydrate soln, or 20–30 min for the acidified chloral hydrate glycerol soln)

(b) *Sweetened chocolate and cocoa* —If the sample contains sugar, remove the sugar by washing the defatted material several times with H O and finally with a mixture (equal volumes) of alcohol and ether Dry, powder, and mix the sample thoroly Weigh 2 mg, transfer to the ruled slide, and proceed as directed under (a)

Since it has been shown that sugar does not interfere with the determination, some analysts prefer not to remove sugar, in which case 5 mg of defatted material is taken and the results calculated to the basis of 2 mg of fat and sugar free material

34 EXAMINATION

Examine the entire mount and count all fragments of stone cell tissue present Compare the results with those obtained on standard samples containing known percentages of cacao shell Report results on the fat free basis

CRUDE FIBER—OFFICIAL FIRST ACTION

(For cacao products except milk chocolate)

35 Treat 7 g of liquor or 50 g of sweet chocolate in a nursing bottle with 100 cc of ether, centrifugalize, and decant the supernatant liquor twice. Dry the residue in an oven at about 100° and then powder in the bottle with a flattened glass rod. (In some cases it may be necessary to grind the material in a mortar and extract a third time with ether.) Wash the mixture in the nursing bottle with three 100 cc portions of H_2O at room temp., shaking well each time, until no cocoa material adheres to the bottle. Centrifugalize after each washing for 10–15 min., and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc portions of 95% alcohol and one 100 cc portion of ethyl ether. Transfer the residue to a Pt dish, dry to constant weight, and grind in a mortar. Weigh 2 g of the dried material and determine the percentage of crude fiber as directed under **XXVII, 19**, using linen for both acid and alkaline filtrations. Calculate the percentage of crude fiber on moisture fat and sugar free basis (E) by the formula $E = 0.7D$.

CRUDE FIBER—TENTATIVE

36 (For milk chocolate)

Proceed as directed under **XXVII, 19**, using sufficient sample prepared as directed under **1** to contain approximately 1 g of water-, sugar-, and fat free material, making both filtrations thru paper and weighing the washed fiber either upon a weighed filter in the usual way, or rinse from the paper into a weighed Gooch, dry, and weigh.

STARCH

Direct Acid Hydrolysis Method—Tentative

37 Weigh 4 g of the sample if unsweetened or 10 g if sweetened, into a small porcelain mortar, add 25 cc of ether, and grind. After the coarser material has settled, decant the ether, together with the fine suspended matter on an 11 cm paper of sufficiently fine texture to retain the crude starch. Repeat this treatment until no more coarse material remains. After the ether has evaporated from the filter, transfer the fat free residue to the mortar by means of a jet of cold H_2O and rub to an even paste, filtering on the paper previously used. Repeat this process until all the sugar is removed. In the case of sweetened products the filtrate should measure at least 500 cc. Determine crude starch in the extracted residue as directed under **XXVII, 23**.

Diastase Method—Tentative

38 Remove fat and sugar from 1 g of the sample if unsweetened or 10 g if sweetened as directed under **34**. Carefully wash the wet residue into a beaker with 100 cc of H_2O, heat to boiling over asbestos with constant stirring, and continue the boiling and stirring for 30 min. Replace the H_2O lost by evaporation and immerse the beaker in a water bath kept at 55–60°. When the liquid has cooled to the temp. of the bath add 20 cc of freshly prepared malt extract (**XXVII, 26**) and digest the mixture for 2 hours with occasional stirring. Boil a second time for 30 min., dilute cool, and digest as before with another 20 cc portion of the malt extract. Heat again to boiling, cool, and transfer to a 250 cc flask. Add 3 cc of alumina cream, dilute to the mark, and filter thru a dry paper. The residue on the paper should show no signs of starch when

examined microscopically Continue from this point as directed under XXVII, 25, beginning with the words "Place 200 cc of the filtrate in a flask, add 20 cc of HCl (sp gr 1 125) "

39 COLORING MATTERS—TENTATIVE

Proceed as directed under XXI, 2(e)

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[4] Ibid , 13, 45 (1930)
[5] Ibid , 5, 263 (1921), 7, 150 (1923)
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[8] Ibid 14 (1931)

XX CEREAL FOODS

WHEAT FLOUR[1]

DIRECTIONS FOR SAMPLING—OFFICIAL

1 Sample a number of sacks equivalent to the square root of the number in the lot, but not less than 10, i e , 10 from 100 or less, 15 from 225, 20 from 400 sacks, etc

Select the sacks to be sampled according to their exposure in the ratio of 4 from the most exposed, 3 from the next less exposed, 2 from the next, and 1 from the least exposed portion of the lot

From each sack to be sampled, draw a core from one corner of the top diagonally to the center of the sack by means of a cylindrical pointed, polished metal trier ½ inch in diameter, with a slit at least ¾ the circumference Draw a second core from the other top corner to ¾ the distance to the center of the sack

Deliver the 2 cores at once to a clean, dry, air tight container which has stood open for a few minutes near the lot of flour to be sampled and seal immediately Use a separate container for each sack sampled One of the following containers may be used (1) A pint fruit jar provided with a rubber gasket, (2) a rubber pouch which can be tied or sealed to exclude moisture or air, (3) a tin can or box with a moisture and air tight friction top

Before opening the sample for analysis, alternately invert and roll each container 25 times or more if necessary, to secure a homogeneous mixture Avoid extreme temp and humidities when opening the containers for analysis Keep the sample tightly sealed at all other times

TOTAL SOLIDS (MOISTURE INDIRECT METHOD)

I Vacuum Method[2]*—Official*

APPARATUS

2 (a) *Metal dish* —Diameter about 55 mm height about 15 mm, provided with an inverted slip in cover fitting tightly on inside

(b) *Air tight desiccator* —Should contain reignited quick lime or CaC_2

(c) *Vacuum oven* —Connect with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm or less of Hg and provided with a thermometer passing into the oven in such a way that the bulb is near the samples Connect a H_2SO_4 gas drying bottle with the oven for admitting dry air when releasing the vacuum

(d) *Mercury manometer* —Used to indicate the pressure of the partial vacuum

DETERMINATION

3 Weigh accurately about 2 g of the well mixed sample in a covered dish that previously has been dried at 98–100° cooled in the desiccator and weighed soon after attaining room temp Loosen the cover (do not remove) and heat at 98–100 to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm or less of Hg Admit dry air into the oven to bring to atmospheric pressure Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temp is attained Report the flour residue as total solids and the loss in weight as moisture (indirect method)

II Air Oven Method[3]—*Official*

(This method gives results closely approximating those obtained by the vacuum method)

4 APPARATUS

(a) *Metal dish and desiccator* —Described under 2(a)

(b) *Oven* —Maintained at approximately 130° (±3°) and provided with an opening for ventilation

(c) *Thermometer* —Place with its bulb near the samples

5 DETERMINATION

Weigh accurately approximately 2 g of the well mixed sample in a covered dish that has been dried previously at approximately 130° (±3°), cooled in the desiccator, and weighed soon after attaining room temp Uncover the sample and dry the dish, cover, and contents in the oven at approximately 130° (±3) for 1 hour Cover the dish while still in the oven, transfer to the desiccator, and weigh soon after room temp is attained Report the flour residue as total solids and the loss in weight as moisture (indirect method)

6 ASH[4]—OFFICIAL

Weigh 3–5 g of the well mixed sample into a shallow, relatively broad ashing dish, which has been ignited, cooled in a desiccator, and weighed soon after attaining room temp Incinerate in a furnace at approximately 550° (dull red) until a light gray ash results or until no further loss in weight occurs Cool in the desiccator and weigh soon after room temp is attained Reignited quick lime or CaC is a satisfactory drying agent for the desiccator

7 CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Proceed as directed under XXVII, 15 With fine flour the addition of an equal weight of clean, dry sand may be necessary

8 FAT (ACID HYDROLYSIS METHOD)[5]—OFFICIAL

Place 2 g of the flour in a 50 cc beaker, add 2 cc of 95% alcohol, and stir so as to moisten all particles (The moistening of the sample with alcohol prevents lumping on addition of the acid) Add 10 cc of HCl (25+11), mix well set the beaker in a water bath held at 70–80 , and stir at frequent intervals for 30–40 min Add 10 cc of 95% alcohol and cool Transfer the mixture to a Rohrig or Mojonnier fat extraction apparatus Rinse the beaker into the extraction tube with 25 cc of ethyl ether in 3 portions and shake the mixture well Add 25 cc of redistilled petroleum ether (b p below 60°) and mix well Let stand until the upper liquid is practically clear Draw off as much as possible of the ether fat soln thru a filter consisting of a pledget of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether into a weighed 125 cc beaker-flask containing some porcelain chips or broken glass Before weighing the beaker flask dry it in a drying oven at 98–105° and then allow it to stand in the air to constant weight Re extract the liquid remaining in the tube twice, each time with only 15 cc of each ether Shake well on the addition of each ether Draw off the clear ether solns thru the filter into the same flask as before and wash the tip of the spigot, the funnel, and end of the funnel stem with a few cc of a mixture of the 2 ethers in equal volumes free from suspended H_2O Evaporate the ethers slowly on a steam bath, then dry the fat in a drying oven at 90–105° until it ceases to lose weight (approximately 75 min) Remove the flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh

(Owing to the size of the flask and the nature of the material, there is less error by cooling in air than in a desiccator) Correct this weight by a blank determination on the reagents used

CRUDE FIBER—OFFICIAL

9 Proceed as directed under XXVII, 17–19

ACIDITY OF WATER EXTRACT—TENTATIVE

10 Weigh 18 g of the flour into a 500 cc Erlenmeyer flask and add 200 cc of CO_2 free H_2O Keep the flask, loosely stoppered, for an hour in a water bath maintained at 40 , shaking occasionally Filter thru a dry folded filter, returning the first 10–15 cc of the filtrate to the filter Titrate 100 cc of the clear filtrate with 0 05 *N* NaOH soln, using phenolphthalein indicator 1 cc of 0 05 *N* NaOH soln = 0 05% acidity as lactic acid

HYDROGEN ION CONCENTRATION—OFFICIAL FIRST ACTION

11 Weigh 10 g of flour (or some multiple thereof) into a clean, dry Erlenmeyer flask and add for each 10 g of flour 100 cc of distilled H_2O at a temp of 25° Shake or whirl the flask until the particles of flour are evenly suspended and the mixture is free from lumps Place in a thermostat at 25° and shake, continuously or intermittently in such a manner as to keep the flour particles in suspension, for 30 min Let stand quietly for 10 min , then decant the supernatant liquid into a suitable vessel and immediately determine its hydrogen ion concentration electrometrically, using electrodes and a potentiometric set up that have been checked thru the use of a buffer soln of known hydrogen ion concentration

SUGARS—TENTATIVE

12 Determine reducing sugars and sucrose as directed under XXVII, 21 and 22

PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen)

13 Determine N as directed under II, 19, 22 or 24, and multiply the percentage of N by 5 7 to obtain the percentage of protein Also use the factor 5 7 to convert N to protein in wheat used for manufacturing purposes or human food

70 PER CENT ALCOHOL-SOLUBLE PROTEINS

I By Nitrogen Determination—Tentative

14 Transfer 1 g of the flour to a 150–200 cc bottle or Erlenmeyer flask and add 100 cc of alcohol 70% by volume taking care that none of the material adheres to the bottom of the container Shake thoroly 10–12 times at intervals of 30 min at room temp or shake continuously in a shaking machine for 1 hour and then set aside overnight Shake thoroly once more and filter thru a dry, folded filter returning the first runnings to the filter until a clear filtrate is obtained Pipet 50 cc of the filtrate equivalent to 2 g of the sample, into a Kjeldahl flask dilute with 100 cc of H_2O to prevent frothing during digestion, and determine N as directed under II, 19, 22 or 24 (Make a blank determination on the reagents)

II By Polarization—Tentative

REAGENT

15 *Millon's reagent* —Dissolve metallic Hg in an equal weight of HNO_3 and dilute the soln with an equal volume of H_2O The freshly prepared soln must be used

16 DETERMINATION

Weigh 15 97 g of the flour into a 300 cc flask and add 100 cc of alcohol (sp gr 0 90) Shake at 30 min intervals for 3 hours and then let stand overnight Filter thru a dry, folded filter and polarize in a 200 mm tube Precipitate the proteins in 50 cc of the filtrate by the addition of 5 cc of Millon's reagent Shake filter and polarize the filtrate in a 200 mm tube Multiply the reading in degrees Ventzke by 1 1 to correct for the dilution and deduct the product from the first reading This difference, multiplied by 0 2, gives the percentage of gliadin N [7]

17 PROTEINS SOLUBLE IN 5 PER CENT POTASSIUM SULFATE SOLUTION—TENTATIVE

Weigh 6 g of the flour into a 200 cc flask and introduce exactly 100 cc of 5% K_2SO_4 soln Shake at 30 min intervals for 3 hours or, better, agitate at moderate speed in a mechanical shaker for 1 hour, let settle 30 min , and filter Determine the N in 50 cc of the filtrate as directed under II, 22 or 24, making allowance for the N contained in the reagents

18 GLOBULIN AND ALBUMIN (EDESTIN AND LEUCOSIN) AND AMINO NITROGEN—TENTATIVE

Weigh 10 g of the flour into a 500 cc Erlenmeyer flask, add 250 cc of 1% NaCl soln stopper the flask, and shake thoroly Let stand, with occasional shaking, for 3 hours filter, and evaporate 100 cc of the filtrate to a small volume in a Kjeldahl digestion flask with 5 cc of H_2SO_4 Add 25 cc more acid and determine the N as directed under 19, 22, or 24 To a second 100 cc of the filtrate add 5 cc of 20% phosphotungstic acid soln, shake thoroly, allow to settle, and filter by decantation Wash slightly with H_2O, concentrate the filtrate with 5 cc of H_2SO_4 in a Kjeldahl flask, and determine the amino N as directed under II, 19, 22, or 24 Deduct the amino N from N found in the first fraction to obtain the N as globulin and albumin[8] Make allowance for the N contained in the reagents

GLUTENIN

19 *Method I —Tentative*

Deduct the sum of the K_2SO_4 soluble N, 17, and the alcohol soluble N, 14, from the total organic and ammoniacal N 13, and multiply the difference by 5 7

Method II —Tentative[9]

20 REAGENTS

(a) *Barium hydroxide* —Freshly powdered

(b) *Methyl alcohol* —96%, free from acids, aldehydes, and ketones Synthetic methanol preferred

Flour and reagents should be allowed a minimum exposure to the air at all times

21 DETERMINATION

Weigh 8 g of flour into a 200 cc flask preferably a sugar flask or one that readily permits thoro mixing of the suspension when shaken Add 0 2 g of $Ba(OH)_2$, follow at once with 50 cc of distilled H_2O (CO_2-free), and stopper tightly Shake immediately to form a smooth suspension Let stand for 1 hour at room temp , shaking frequently Add sufficient methyl alcohol to allow 5 cc of liquid above the mark (to correct for volume of flour) when thoroly mixed Shake vigorously for 2 min After the starch settles to the bottom, pour the supernatant liquid *at once* thru a cotton plug, repeating the filtrations 2 or 3 times if necessary Immediately withdraw 50 cc for the Kjeldahl N determination Do not allow more than 15 min to elapse from

the time the methyl alcohol is added to the withdrawal of the 50 cc aliquot because gliadin will begin to precipitate after standing for a short period of time To prevent troublesome foaming add 150–200 cc of H_2O to the Kjeldahl flask before starting the digestion of the alcoholic extract Convert the N to protein by the factor 5 7, subtract the percentage of protein in the extract from the percentage of total protein (N × 5 7) as determined in a separate portion of flour and record the difference as the percentage of glutenin in the flour

CRUDE GLUTEN

Qualitative Test—Tentative

22 Place a very small quantity (about 1 5 mg) of the flour on a microscope slide, add a drop of water containing 0 2 g of water soluble eosin in 1 liter and mix by means of a cover glass holding it at first in such a manner that it is raised slightly above the slide and taking care that none of the flour escapes from beneath it Finally allow the cover glass to rest on the slide and rub it back and forth until the gluten has collected into rolls The operation should be carried out on a white paper so that the formation of gluten rolls can be noted Wheat flour or other flours containing gluten show by this treatment a copious quantity of gluten which absorbs the eosin with avidity, assuming a carmine color Rye flour and corn flour yield only a trace of gluten buckwheat flour, no appreciable quantity If the flour is coarse or contains a considerable quantity of bran elements as is true of buckwheat flour and low-grade wheat flour the test should be made after bolting as the bran particles and coarse lumps interfere with the formation of gluten rolls

Quantitative Method—Tentative

23 (Results are approximate)

Weigh 20 g of the flour into a cup or porcelain mortar add sufficient tap H_2O (about 15 cc) to form a firm dough ball and work into a dough with a spatula or pestle, taking care that none of the material adheres to the utensil After allowing the dough to stand in H_2O at room temp for an hour, knead gently in a stream of tap H_2O until the starch and all soluble matters are removed Do this operation which requires approximately 12 min over bolting cloth To determine whether or not the gluten is starch free let 1 or 2 drops of the wash H_2O obtained by squeezing the gluten fall into a beaker containing perfectly clear water If starch is present a cloudiness appears Allow the gluten thus obtained to stand in H_2O for an hour, press as dry as possible between the hands roll into a ball place in a weighed flat bottomed dish and weigh as moist gluten Transfer to an oven dry to constant weight at 100 (about 24 hours) cool and weigh as dry gluten or heat the moist gluten at approximately 200 for 1 20 min, or until the puffed gluten ball has become firm Then dry to constant weight in a drying oven

WATER SOLUBLE PROTEIN NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL — TENTATIVE

REAGENTS

24 (a) Alcohol—40% Mix 40 volumes of H_2O and 60 volumes of 95% ethyl alcohol

(b) Tartaric acid—Ignite and pulverize an 8 mesh sieve

DETERMINATION

25 Place 20 g of the sample in an 8 oz nursing bottle add 100 cc of H_2O from a pipet shake the bottle to prevent lumping of the sample and add exactly 100 cc more H_2O Mix the contents of the bottle gently by hand or on a slowly re-

16 DETERMINATION

Weigh 15 97 g of the flour into a 300 cc flask and add 100 cc of alcohol (sp gr 0 90) Shake at 30 min intervals for 3 hours and then let stand overnight Filter thru a dry, folded filter and polarize in a 200 mm tube Precipitate the proteins in 50 cc of the filtrate by the addition of 5 cc of Millon's reagent Shake filter and polarize the filtrate in a 200 mm tube Multiply the reading in degrees Ventzke by 1 1 to correct for the dilution and deduct the product from the first reading This difference, multiplied by 0 2, gives the percentage of gliadin N [7]

17 PROTEINS SOLUBLE IN 5 PER CENT POTASSIUM SULFATE SOLUTION—TENTATIVE

Weigh 6 g of the flour into a 200 cc flask and introduce exactly 100 cc of 5% K_2SO_4 soln Shake at 30 min intervals for 3 hours or, better, agitate at moderate speed in a mechanical shaker for 1 hour, let settle 30 min , and filter Determine the N in 50 cc of the filtrate as directed under II, 22 or 24, making allowance for the N contained in the reagents

18 GLOBULIN AND ALBUMIN (EDESTIN AND LEUCOSIN) AND AMINO NITROGEN—TENTATIVE

Weigh 10 g of the flour into a 500 cc Erlenmeyer flask, add 250 cc of 1% NaCl soln stopper the flask, and shake thoroly Let stand, with occasional shaking, for 3 hours, filter, and evaporate 100 cc of the filtrate to a small volume in a Kjeldahl digestion flask with 5 cc of H_2SO_4 Add 25 cc more acid and determine the N as directed under 19, 22, or 24 To a second 100 cc of the filtrate add 5 cc of 20% phosphotungstic acid soln, shake thoroly, allow to settle, and filter by decantation Wash slightly with H_2O, concentrate the filtrate with 5 cc of H_2SO_4 in a Kjeldahl flask, and determine the amino N as directed under II, 19, 22, or 24 Deduct the amino N from N found in the first fraction to obtain the N as globulin and albumin[8] Make allowance for the N contained in the reagents

GLUTENIN

19 *Method I —Tentative*

Deduct the sum of the K_2SO_4 soluble N, 17, and the alcohol soluble N, 14, from the total organic and ammoniacal N, 13, and multiply the difference by 5 7

Method II —Tentative[9]

20 REAGENTS

(a) *Barium hydroxide*—Freshly powdered

(b) *Methyl alcohol*—96%, free from acids, aldehydes and ketones Synthetic methanol preferred

Flour and reagents should be allowed a minimum exposure to the air at all times

21 DETERMINATION

Weigh 8 g of flour into a 200 cc flask, preferably a sugar flask or one that readily permits thoro mixing of the suspension when shaken Add 0 2 g of $Ba(OH)_2$ follow at once with 50 cc of distilled H_2O (CO_2 free), and stopper tightly Shake immediately to form a smooth suspension Let stand for 1 hour at room temp , shaking frequently Add sufficient methyl alcohol to allow 5 cc of liquid above the mark (to correct for volume of flour) when thoroly mixed Shake vigorously for 2 min After the starch settles to the bottom, pour the supernatant liquid *at once* thru a cotton plug repeating the filtrations 2 or 3 times if necessary Immediately withdraw 50 cc for the Kjeldahl N determination Do not allow more than 15 min to elapse from

the time the methyl alcohol is added to the withdrawal of the 50 cc aliquot, because gliadin will begin to precipitate after standing for a short period of time To prevent troublesome foaming add 150–200 cc of H_2O to the Kjeldahl flask before starting the digestion of the alcoholic extract Convert the N to protein by the factor 5 7, subtract the percentage of protein in the extract from the percentage of total protein (N×5 7) as determined in a separate portion of flour and record the difference as the percentage of gluten in the flour

CRUDE GLUTEN

22 *Qualitative Test[13]—Tentative*

Place a very small quantity (about 1 5 mg) of the flour on a microscope slide add a drop of water containing 0 2 g of water soluble eosin in 1 liter and mix by means of a cover glass, holding it at first in such a manner that it is raised slightly above the slide and taking care that none of the flour escapes from beneath it Finally allow the cover glass to rest on the slide and rub it back and forth until the gluten has collected into rolls The operation should be carried out on a white paper so that the formation of gluten rolls can be noted Wheat flour, or other flours containing gluten, show by this treatment a copious quantity of gluten, which absorbs the eosin with avidity, assuming a carmine color Rye flour and corn flour yield only a trace of gluten, buckwheat flour, no appreciable quantity If the flour is coarse or contains a considerable quantity of bran elements, as is true of buckwheat flour and low-grade wheat flour the test should be made after bolting as the bran particles and coarse lumps interfere with the formation of gluten rolls

23 *Quantitative Method—Tentative*

(Results are approximate)

Weigh 20 g of the flour into a cup or porcelain mortar, add sufficient tap (about 15 cc) to form a firm dough ball and work into a dough with a spat pestle, taking care that none of the material adheres to the utensil After the dough to stand in H_2O at room temp for an hour, knead gently in a stream tap H_2O until the starch and all soluble matters are removed Do this oper which requires approximately 12 min over bolting cloth To determine whether not the gluten is starch free let 1 or 2 drops of the wash H_2O, obtained by ing the gluten, fall into a beaker containing perfectly clear water If starch is ent a cloudiness appears Allow the gluten thus obtained to stand in H_2O for an hour, press as dry as possible between the hands, roll into a ball place in a weighed, flat bottomed dish, and weigh as moist gluten Transfer to an oven, dry to constant weight at 100° (about 24 hours), cool, and weigh as dry gl or heat the moist gluten at approximately 230° for 15–20 min , or until the gluten-ball has become firm Then dry to constant weight in a

WATER SOLUBLE PROTEIN NITROGEN PRECIPITABLE TENTATIVE

24 REAGENTS

(a) *Alcohol*—40% Mix 40 volumes of H_2O and 35

(b) *Asbestos*—Ignite and rub thru an 8-mesh sieve.

25 DETERMINATION

Place 20 g of the sample in an 8 oz nursing bottle, add pet, shake the bottle to prevent lumping of the sample, and H_2O Mix the contents of the stoppered bottle gently by

volving wheel for 1 hour (The temp of the H_2O should not exceed 30°) Centrifu galize to *facilitate filtration and filter thru a thin asbestos pad* in a Hirsch funnel, using light suction Determine N in 50 cc of the filtrate as directed under II, 19, 22 or 24, distilling the NH_3 into 20 cc of 0 1 N acid Run a blank on the reagents Pipet off 100 cc of the above filtrate into a 200 cc volumetric flask, add 15 cc of NaCl soln (28 g diluted to 300 cc), fill nearly to the mark with 95% alcohol, mix carefully to avoid foaming, cool to room temp , make up to the mark with alcohol, mix well, and allow to stand overnight Pipet off the supernatant liquid and filter thru an 18½ cm fluted filter paper Determine N in 100 cc of the filtrate as above (In order to avoid bumping it is advisable to add the H_2SO_4 and boil off the alcohol before adding the K_2SO_4 and HgO) Subtract the value obtained from the water soluble N to obtain the water soluble N precipitable by 40% alcohol

26 LIPOIDS[28]—OFFICIAL

Add 15 cc of alcohol, 70% by volume, to 5 g of the flour in a 200 cc nursing bottle Give the bottle a gentle rotary motion so as to moisten all the particles with the alcohol stopper and set in a water bath kept at 75–80° Heat for 15 min with frequent mixing by the same rotary motion Add 27 cc of 95% alcohol, stopper the bottle, and shake vigorously for 2 min Cool, add 45 cc of ether, and shake well for 5 min (The sample should now be in a fine state of division) Centrifugalize just sufficiently to throw the solid particles out of suspension but not so as to pack the sample too firmly Decant the liquid into a 250 cc beaker containing some bits of broken porce*lain or glass, and rinse off the bottle neck* with ether Re-extract the sample with 3 successive 20 cc portions of ether, shake 1 or 2 min each time, centrifugalize, and decant into the beaker containing the first extract Evaporate the combined ether-alcohol extracts just to dryness on the steam bath Drive off any remaining moisture on the sides of the beaker by placing in a drying oven at 98–105° for 5 min Dissolve the dry extract in approximately 15 cc of $CHCl_3$ and filter the soln into a previously dried and weighed Pt dish thru a pledget of cotton packed in the stem of a funnel Free with a glass rod any solid extract adhering to the beaker and transfer thru the filter into the first washings by means of $CHCl_3$ from a wash bottle all extract from the beaker bottom and sides Finally wash the funnel and stem tip The filtrate should be perfectly clear Evaporate the $CHCl_3$ on a steam bath and dry the dish and contents in a drying oven at 98–105° until no more weight is lost (75–90 min) Weigh Report the extract as lipoids

27 LIPOID PHOSPHORIC ACID[29] (P O)—OFFICIAL

APPARATUS

29 *Extraction cylinder*—Glass stoppered, graduated at 40 cc, 80 cc and 130 cc, and of the following dimensions diameter about 1¾ inches, height about 12 inches

DETERMINATION

30 Place the extract from 5 g of flour prepared as directed under 26 in a 200 cc Erlenmeyer flask, add 5 cc of 50% aqueous KOH, and boil the mixture for 1 hour under a reflux condenser Transfer to the extraction cylinder and wash to the 40 cc mark with redistilled 95% alcohol Complete the transfer first with warm then with cold H_2O, until the total volume is 80 cc Rinse the flask with 50 cc of petroleum ether and add the rinsings to the contents of the cylinder previously cooled to room temp Shake as vigorously as possible for 1 min and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 cc Draw off the petroleum ether layer as closely as possible by means of a slender glass siphon into a separatory funnel of 500 cc capacity Repeat the extraction at least 6 more times, using 50 cc of petroleum ether for each extraction Wash the combined extracts into a separatory funnel 3 times with 20 cc portions of 10% alcohol by volume, shaking vigorously each time Transfer the petroleum ether extract to a weighed Erlenmeyer flask (the flask should be dried at 100–105°, cooled in a desiccator then brought to equilibrium with the atmosphere and weighed), distil, or if desired evaporate the petroleum ether on a steam bath in a current of air Heat the flask (lying on its side) with residue until a constant weight is obtained in an oven at a uniform temp not less than 100° nor more than 110° (It is important to displace with air any residue vapors of petroleum ether remaining in the flask after heating and before it is weighed) Deduct any blank from the weight before calculating unsaponifiable matter Test the final residue for solubility in 50 cc of petroleum ether at room temp Filter and wash free from the insoluble residue if any Evaporate and dry in the same manner as before

II Modified Kerr Sorber Method—*Tentative*

REAGENTS AND APPARATUS

31 (a) *Potassium hydroxide soln*—100 g of KOH dissolved in 100 cc of H_2O

(b) *Potassium hydroxide soln*—Approximately 0 2 *N* 11 2 g of KOH dissolved in 1000 cc of H_2O

(c) *Ethyl alcohol*—Approximately 95% by volume

(d) *Ethyl ether*—U S P

(e) *Phenolphthalein soln*—1 g of phenolphthalein dissolved in 100 cc of 95% alcohol

APPARATUS

32 (a) *Separatory funnel*—500 cc capacity ether tight Lubricate the glass connections with H_2O

(b) *Erlenmeyer flask or beaker flask for saponification*—100–200 cc capacity

(c) *Erlenmeyer flask or beaker flask*—250 cc capacity

PROCEDURE

33 Place the extract from 5 g of flour prepared as directed under 26 in the saponification flask Add 3 cc of the concentrated KOH soln Place a small, short stemmed funnel in the neck of the flask to serve as a condenser Boil gently on the steam bath for about 20 min , or until complete saponification occurs Cool to about 30 , add 50 cc of ether, mix, and transfer to the separatory funnel Rinse the flask with 2

successive 50 cc portions of ether, add to the separatory funnel, and mix thoroly Wash the saponification flask (b) with 100 cc of the dilute KOH soln and pour into the separatory funnel in a slow, steady stream Rotate the funnel very gently to secure better contact of the solns but do not shake (Shaking at this stage brings about stubborn emulsion) Allow the liquids to separate completely and then slowly draw off as much of the soap soln as possible Do not draw off any layer of emulsion that may be formed Keep the volume of the ether at about 150 cc by replacing that dissolved by the wash solns Further treat the ether soln with 2 successive 100 cc portions of the alkaline wash soln in the manner described previously Add 30 cc of H O to the ether and rapidly rotate the liquid layers When the layers have separated completely, draw off the H_2O, repeating this treatment until the washings are free from alkali, as shown by testing with phenolphthalein (Three washings usually suffice) Transfer the ether soln quantitatively thru a pledget of cotton in the stem of a funnel to the weighed 250 cc Erlenmeyer flask or beaker flask Before weighing the flask dry it in an oven at about 100°, and then allow it to stand in the air to constant weight Distil off the ether and dry the flask and residue at about 100° until no further loss in weight occurs Allow the flask with unsaponifiable matter to come to equilibrium with the atmosphere before weighing Deduct from the weight of the unsaponifiable matter any blank obtained from the reagents used

34 COLD WATER-SOLUBLE EXTRACT—TENTATIVE

Weigh 20 g of the flour into a 500 cc Erlenmeyer flask and add gradually 200 cc of H_2O at a temp not higher than 10° Shake vigorously when about 50 cc of H_2O has been added and continue shaking during the addition of the remainder Allow to stand at 10° for 40 min , shaking occasionally Filter rapidly, returning the first runnings to the filter, until a clear filtrate is obtained Pipet 20 cc of the clear filtrate into a weighed dish, evaporate to dryness on a steam bath, and dry in an oven at about 100° for periods of 30 min to constant weight

STARCH[a]—TENTATIVE

35 REAGENT

Hydrochloric acid —Mix approximately equal volumes of HCl and H_2O and adjust so that 100 cc of this mixture contains 20 5–21 0 g of HCl

36 DETERMINATION

Weigh accurately a sufficient quantity of the finely grou[nd] 0 5–1 0 g of starch Transfer to a funnel fitted with a 9 cm, S and[ample to repr]or Whatman No 40 filter paper and extract 4 times with each o[f 589 white ri]vents in the order named ether, 70% (by volume) alcohol, and [the following] drained filter and contents to a 50 cc beaker Add 1 cc of the cold [water Transf]the material with a stirring rod having a flattened end, and conti[nue adding the] gradually, with constant tamping and stirring until the filter pa[per and samp]disintegrated to a smooth suspension Transfer to a 100 cc wide-n[ecked volum]flask, rinsing with the HCl reagent from a wash bottle Fill to [the mark with] same acid and then add 0 5 cc more to compensate for the volu[me occupied by] filter paper Mix and allow to stand for 3–5 min , shaking occa[sionally.] treatment with acid the temp should not exceed 22° Filter thru a Gooch crucible prepared with a dry mat of ignited asbestos and filled ⅔ full with dry, fluffy, ignited asbestos Pipet 50 cc of the filtrate into a 200 cc beaker (tall form) containing 115 cc of 95% (by volume) alcohol (To prevent hydrolysis this last step must be completed within 35 min of the initial contact of the acid with the starch) Allow the pipet

to deliver completely and then stir with a whipping motion for 1 min to flocculate the precipitated starch Allow to stand for not longer than 5 min and then decant the supernatant liquid which is somewhat turbid, thru a weighed Gooch crucible that has been fitted with a thin pad of ignited asbestos Wash the precipitate by decantation, using two successive 15 cc portions each of 70% (by volume) and 95% (by volume) alcohol, breaking up the precipitate with a stirring rod during each washing Decant each portion thru the crucible and finally transfer the starch completely by means of a jet of 95% (by volume) alcohol Dry the crucible and contents to constant weight which requires about 1½ hours at 130° or 5 hours in a vacuum oven at about 100° Allow the crucible to remain uncovered during drying but replace the cover before removing the crucible from the oven, because the starch is extremely hygroscopic Dry in a desiccator charged with P_2O_5, CaC_2, or ignited CaO, cool, and weigh immediately

CHLORINE

37 *Qualitative Test (Chlorine Bleached Flours)—Tentative*

Extract 30 g of the flour with gasoline and allow the solvent to evaporate A small quantity of oil remains Heat a piece of Cu wire in a colorless gas flame until it is black and no longer colors the flame green Dip the hot end of the wire into the oil and again bring into the flame If Cl or Br has been used as a bleaching agent, a green or blue coloration is produced

QUANTITATIVE METHODS

Method I—Tentative

38

Weigh 20 g of the flour into a flat bottomed Al dish, 8–10 cm in diameter, and dry 5 hours in a drying oven at 98–105°, transfer with as little exposure to the air as possible, to a continuous fat extractor, and extract for 16 hours with anhydrous alcohol free ether that is also free from Cl Transfer the ether extract to a Pt dish and add 25 cc of a soln containing 25 g of NaOH or KOH and 15 g of $NaNO_3$ per liter Place the dish on a steam bath, evaporate to dryness and ignite in a muffle at a dull red heat until the contents are thoroly charred Extract the charred mass with 25 cc of 1% HNO_3 and filter Return the residue to the dish, char, and again extract with 25 cc of 1% HNO_3 Filter, wash with hot H_2O, return to the dish, and ignite to a white ash Dissolve the ash in 5% HNO_3 and add the soln to the filtrates previously obtained Determine the Cl in the combined filtrates either gravimetrically as directed under XII, 33, or volumetrically as directed under XII, 35, using 0 02 *N* solns for greater accuracy

Special precautions should be taken that the air of the laboratory during the entire operation is not contaminated by Cl or HCl fumes and that all reagents employed are as free as possible from Cl In all cases a blank determination should be conducted at the same time and a correction introduced if necessary Report results in parts of Cl per million of flour

Method II[a]—Tentative

REAGENTS

39

(a) *Petroleum ether fractioned at 60–100* —Do not use low boiling petroleum ether because of errors introduced thru rapid evaporation

(b) *Alcoholic sodium hydroxide soln* —Dissolve 40 g of NaOH in 1 liter of 95% alcohol

(c) *Potassium chromate indicator* —Dissolve 5 g of K_2CrO_4 in H_2O add the $AgNO_3$ soln (d) until a slight red precipitate is produced, filter, and dilute to 100 cc

(d) *Standard silver nitrate soln* —Dissolve 4 791 g of $AgNO_3$ in H_2O and dilute to 1 liter 1 cc = 1 mg of Cl Check by titration against a standardized soln of NaCl

40 DETERMINATION

Weigh 75 g of the flour into a cork stoppered bottle and add from a pipet 150 cc of the petroleum ether Stopper tightly and shake vigorously for about 1 min Allow to stand 1 hour, again shake until the flour particles are in suspension, and then set aside overnight Shake once more to suspend the flour particles, allow to settle for a few min , and filter thru a dry folded filter (The funnel and receiving flask should be covered to reduce evaporation during filtration) Pipet 50 cc of the filtrate into a Pt dish of about 80 cc capacity If a Pt dish of this size is not available, evaporate the 50 cc of filtrate to a small volume in a porcelain evaporating dish on a steam bath and carefully transfer the fatty concentrate to a small Pt dish, washing out the last traces of fat with several portions of petroleum ether Add 5 cc of the alcoholic NaOH soln and evaporate to dryness on a steam bath Char carefully in a muffle at low redness Extract the charred mass with 2 successive 20 cc portions of HNO_3 (1+3), being careful to avoid mechanical losses due to evolution of CO_2 Filter these extracts thru a 7 cm quantitative filter paper into a 300 cc flask Then extract the mass 2 or 3 times with H_2O, filtering each portion thru the same 7 cm paper Return this filter paper to the Pt dish containing the charred residue and ignite to a white ash in a muffle furnace Dissolve the ash in HNO_3 (1+16) and add to the soln already obtained Neutralize the acidity with a slight excess of dry $CaCO_3$ add 5 cc of the K_2CrO_4 indicator, and titrate with the standard $AgNO_3$ soln At the same time and under the same conditions prepare and conduct a blank containing the quantity of all reagents used in the determination Since $CaCO_3$ commonly contains appre ciable quantities of chlorides, a definite weighed quantity of this reagent should be employed in each determination and the same quantity used in the blank Correct the buret reading by the number of cc of the standard $AgNO_3$ soln required to give in the blank the shade obtained at the end of the titration of the sample, using in both sample and blank 5 cc of the K_2CrO_4 indicator Report results in parts of Cl per million of flour

Since the quantity of Cl involved in this determination is relatively small, care should be taken to insure that the laboratory atmosphere is as free from Cl as pos sible

Method III[18]*—Tentative*

41 REAGENTS

(a) *Alcohol* —70% by volume Mix 73 volumes of 95% alcohol with 27 volumes of H_2O

(b) *Alcohol* —95% by volume

(c) *Ethyl ether*

(d) *Petroleum ether*

(e) *Alcoholic soda* —To 95% alcohol add metallic Na cut into small pieces in the proportion of 40 g of Na to 1,000 g of alcohol

(f) *Nitric acid* —(2+1) Dilute 500 cc of strong acid to 750 cc with H O

(g) *Silver nitrate soln* —0 005 *N*

(h) *Potassium thiocyanate* —0 005 N

(i) *Ferric ammonium alum soln* —To a cold saturated soln of ferric ammonium alum add enough HNO_3 to cause the disappearance of the brown color

DETERMINATION

42 Weigh 20 g of flour into a 500 cc Erlenmeyer flask add 60 cc of 70% alcohol place the flask upon a steam bath, and heat gently (water should steam but not boil), at the same time rotating the flask until the flour and liquid form a uniform mixture Add 60 cc of 95% alcohol Stopper the flask and shake thoroly for 2 min Allow to cool Add 75 cc of ethyl ether and shake the flask thoroly, then add 150 cc of petroleum ether, and again shake the flask thoroly Pour the entire liquid contents into a separatory funnel being careful to avoid as far as possible transference of any flour particles Add to the flask containing the flour, 40 cc of petroleum ether shake thoroly, and pour the contents into the separatory funnel Repeat with another 40 cc portion of petroleum ether Wash the solvents twice with H_2O, using 30 cc of H_2O the first time and 10–12 cc the second time shake thoroly each time and allow to stand until two sharply defined layers of liquid are formed Run the washed sol vents into a large evaporating dish (or beaker), add 10 cc of the alcoholic soda soln and evaporate to about 10–15 cc Pour this liquid into a 50 cc Pt dish and wash out the evaporating dish with small portions of 95% alcohol until all the liquid and residue have been transferred to the Pt dish Evaporate the contents of the dish to dryness on the steam bath and place the dish with the residue over a small yellow flame of a Bunsen burner Char the residue but do not heat even to low redness be cause the alkali may react with the Pt Allow to cool and add a small quantity of H_2O and 5 cc of HNO_3 (2+1) Boil, and then pour thru an ashless filter paper (12½ cm in diameter), catching the filtrate in a sugar flask calibrated at 100 cc and 110 cc Again boil the residue with a small quantity of H_2O and filter Remove the filter paper, fold once and place in a Pt dish, and heat to low redness until practically all the paper and residue have been reduced to a gray ash (Apply low heat to prevent volatilization of chlorides) Add a small quantity of H_2O and filter Remove the filter boil, and filter Again boil once or twice with small quantities of H_2O filtering as before Add to the liquid in the sugar flask 25 cc of 0 000 N $AgNO_3$ soln, add H_2O to bring the liquid approximately to the 100 cc mark and place the flask in boiling H_2O for about 5 min Remove and allow to cool to room temp Bring the liquid exactly to the 110 cc mark by adding H_2O stopper the flask and mix the contents well Filter thru a dry fine-pore filter paper (12 5 cm in diameter) and return the first portion of the filtrate to the original soln Continue to refilter until the filtrate is entirely clear, and thus secure 100 cc of filtrate Transfer the entire 100 cc to a white porcelain casserole add 2 cc of ferric ammonium alum soln and titrate with 0 005 N KCNS until a permanent light brown coloration appears Deduct the blank determined on all the reagents used and calculate results to the dry basis

NITRITE NITROGEN—TENTATIVE

REAGENTS

43 (a) *Sulfanilic acid soln* —Dissolve 0 5 g of sulfanilic acid in 150 cc of 20% acetic acid

(b) *Alpha naphthylamine hydrochloride soln* —Dissolve by heating 0 2 g of the salt in 1 0 cc of 20% acetic acid

(c) *Standard nitrite soln* —Dissolve 0 1097 g of dry $AgNO_2$ in about 20 cc of hot H_2O add 0 10 g of NaCl shake until the AgCl flocculates and dilute to 1 liter Draw off 10 cc of the clear soln and dilute to 1 liter 1 cc of the last soln = 0 0001 mg of N as nitrite [cf XXXVII, 14 (c)]

The $AgNO_2$ may be prepared as follows To a cold soln of about 2 g of NaNO, or KNO_2 in 20 cc of H_2O add a soln of $AgNO_3$ so long as a precipitate forms Decant the liquid and thoroly wash the precipitate with cold H_2O Dissolve in boiling H_2O

On cooling, the $AgNO_2$ crystallizes out Dry the crystals in the dark at ordinary temp (preferably in a vacuum)

44 DETERMINATION

(1) Select a series of 100 cc volumetric flasks of uniform dimensions and color and place 2 g of high grade, nitrite free flour in each flask, add approximately 70 cc of nitrite free H_2O and shake until the flour is thoroly moistened Add to these flasks varying quantities of the standard $NaNO_2$ soln, so that a series of comparison standards will be obtained having a range covering the probable nitrite content of the unknown sample Reserve one flask for a blank test In order to avoid making a large series of standards it is well to make a preliminary test to ascertain the approximate nitrite content of the unknown If the quantity of nitrite present is small, the nitrite soln in the flasks may be increased by 0 4 cc each If bleaching is excessive, 1 g of flour may be used thruout or the standards may be given a wider variation in nitrite content

To each of 2 similar flasks add 2 g of the flour and 90 cc of H_2O, shake thoroly, digest all the flasks, including the blank, in a water bath at 40° for at least 15 min , and add 2 cc each of the sulfanilic acid and alpha naphthylamine hydrochloride solns to each flask, shaking the mixture after the addition of each reagent Continue the digestion at 40° for an additional 20 min The color must be developed in all the flasks under conditions as nearly uniform as possible Make up to the marks with nitrite free H_2O and compare the unknown with the series of standards This may be done in a large, white enameled pan, the effect of the turbidity due to the flour being minimized by the white background The solns should be allowed to subside and should not be shaken during comparison, or,

(2) Weigh 20 g of the flour into a 500 cc Erlenmeyer flask, add 200 cc of nitrite free H_2O previously warmed to 40 , and close the flask with a rubber stopper Shake vigorously for 5 min and digest for 1 hour in a water bath, keeping the temp of the liquid in the flask at 40 and shaking at 10 min intervals Finally filter thru a nitrite-free filter Return the first runnings to the filter until a clear filtrate is obtained Pipet 50 cc of the filtrate and 50 cc of the standard nitrite soln into small flasks, add to each 50 cc of H_2O and 2 cc each of the sulfanilic acid and alpha naphthylamine hydrochloride solns, shake, and allow to stand 1 hour to bring out the color Compare the two solns in a colorimeter Divide the height of the column of the standard soln by that of the soln of the sample to obtain the parts of nitrous N (free and combined) per million of flour

45 GASOLINE COLOR VALUE—TENTATIVE

Place 20 g of the flour in a wide-mouthed, glass stoppered 120 cc bottle and add 100 cc of colorless gasoline Stopper tightly and shake vigorously for 5 min After standing 16 hours, shake again for a few seconds until the flour has been loosened from the bottom of the bottle and thoroly mixed with the gasoline, then filter immediately thru a dry 11 cm paper into an Erlenmeyer flask, keeping the funnel covered with a watch glass to prevent evaporation In order to secure a clear filtrate, allow a certain quantity of the flour to pass over into the filter, and pass the first portion of the filtrate thru a second time It will be found convenient to fit the filter paper to the funnel by means of H_2O and to dry thoroly either by standing overnight in a well ventilated place or by heating

Determine the color value of the clear gasoline soln in a Schreiner or similar colorimeter, using for comparison a 0 005% K_2CrO_4 soln This soln corresponds to a gasoline number of 1 0 and is conveniently prepared by diluting 10 cc of a 0 5% soln to

1 liter The colorimeter tube, containing the gasoline soln, should first be adjusted so as to read 50 mm then the tube containing the standard chromate soln should be raised or lowered until the shades of yellow in both tubes match The reading of the chromate soln, divided by the reading of the gasoline soln, gives the gasoline color value The color value may be determined also in Nessler tubes using for comparison K_2CrO_4 solns of various dilutions prepared from a 0 5% soln and filling the tubes in all cases to the height of 50 mm

BAKED CEREAL PRODUCTS

BREAD

PREPARATION OF SAMPLE[18]—TENTATIVE

46 (To be used when total solids of original entire loaf is not desired)

Cut the loaf or ½ the loaf of bread into slices 2–3 mm thick Spread the slices on paper and allow them to dry in a warm room until sufficiently crisp and brittle to grind well in a mill Grind the entire sample to pass a 20 mesh sieve, mix well, and keep in an air tight container

TOTAL SOLIDS IN AN ENTIRE LOAF OF BREAD[18]—OFFICIAL

47 Accurately weigh the loaf of bread immediately upon receipt (*A*) Use scales sensitive to at least 0 2 g (When determining whether bread is in conformity with the Department of Agriculture standards do not weigh the loaf sooner than 1 hour after removal from the oven) Should accurate weighing be impossible at this time, seal the sample in an air tight container and accurately weigh as soon thereafter as is practicable (*A*) Preserve the sample in such a manner that no loss of bread solids can occur, whereby the loss would be calculated as moisture Cut the bread into slices 2–3 mm thick (½ of the loaf may be used—official first action) Spread the slices on paper, allow them to dry in a warm room (approximately 15–20 hours), and when apparently dry, break into fragments If the bread is not entirely crisp and brittle allow it to dry longer—until it is in equilibrium with the moisture of the air—in order that no moisture changes may occur during grinding Quantitatively transfer the air dried bread to the scale pan and accurately weigh (*B*) Grind the sample just to pass a 20 mesh sieve, mix well, and keep in an air tight container Determine the percentage of total solids (*C*) of the ground sample as directed under 3 or 5 Calculate total solids of the bread from the formula—

$$TS = \frac{B \times C}{100} \times 100, \text{ or } \frac{B \times C}{A} \text{ in which}$$

A = weight of loaf (or ½ loaf) at time of receipt,

B = weight of the air dried sliced bread, and

C = percentage of total solids in the prepared ground sample

TOTAL SOLIDS OF AIR DRIED GROUND SAMPLE

Method I—Tentative

48 Use 2 g of sample prepared as directed under 46 and proceed as directed under 3

Method II—Official, first action

49 Proceed as directed under 5

50 ASH—OFFICIAL[21]

Use 3–5 g of sample prepared as directed under 46 and proceed as directed under 6

51 CHLORIDES IN ASH—OFFICIAL FIRST ACTION

Proceed as directed under 64

52 PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen)

Determine N as directed under II, 19, 22 or 24, using 2 g of air dried ground sample prepared as directed under 46 Multiply the percentage of N by the factor 5 7 to obtain the percentage of protein

FAT

53 *Method I* [2]*—Official, first action*

Place 5 g of the ground sample in a 200 cc Erlenmeyer flask and add a mixture of 10 cc of 95% alcohol, 2 cc of NH_4OH, and 3 cc of H_2O Place the flask on a steam bath and maintain the contents at the boiling point for 2 min Cool, and extract the mixture with 3 successive 25 cc portions of ether, kneading and tamping the matted material thoroly each time with a glass rod flattened at the end Pour off the ether layer by decantation into a 250 cc beaker, draining off the last 25 cc portion of ether as completely as possible Add another 15 cc portion of the ammoniacal alcoholic soln to the extracted residue in the flask and disintegrate the matted material as thoroly as possible by means of the flattened glass rod, which should be left in the flask for that purpose Return the flask to the steam bath as before, taking care that no loss of material occurs thru bumping due to the presence of ether Boil 2 min Again extract with 3 successive 25 cc portions of ether, adding the ether extracts to those obtained in the first extraction Evaporate the combined extracts to dryness on a steam bath and then extract the fatty residue with 5 or 6 successive 15 cc portions of a mixture of equal volumes of ether and petroleum ether Collect the extracts in a weighed dish (do not try to filter) and evaporate to dryness on a steam bath Dry the residue to constant weight in an oven at the temp of boiling H_2O, cool in a desiccator, and weigh

54 *Method II—Acid hydrolysis method—Official, first action*

Proceed as directed under 8

55 CRUDE FIBER—OFFICIAL FIRST ACTION

Proceed as directed under XXVII, 17–19

EXPERIMENTAL BAKING TEST[23]—TENTATIVE

Basic Procedure

56 EQUIPMENT

Mixing bowl—Ordinary graniteware "oatmeal bowl," top diameter 16 5 cm bottom diameter 5 7 cm, and depth 7 3 cm

Fermentation bowl—Same as mixing bowl but smaller Top diameter 12 7 cm, bottom diameter 5 cm, and depth 5 7 cm

Spatula—Flexible steel blade, approximately 12 7 cm long by 1 9 cm wide

Baking pan—Dimensions Bottom inside, 53 x 93 mm, height (sides), 85 mm, height (ends) 68 mm, top inside (at height of 68 mm) 60 x 105 mm Pans to be made from 4⅄ (0 55 mm) spotless metal, which requires no greasing

Fermentation cabinet and oven—With precise temperature control

Volume measuring apparatus—Calibrate gravimetrically [24] It is suggested that four points of known volumes, namely, 300, 400, 500, and 600 cc, be determined By plotting these four points against their corresponding values, as measured by the apparatus the calibration curve for the apparatus is obtained This calibration likewise corrects for peculiarities of the operator providing such are uniformly and consistently practised

Thermometers

A For fermentation cabinet and dough testing—Use a dough testing thermometer graduated from 15 to 40° or its equivalent on the Fahrenheit scale

B For oven—Use an oven thermometer that has a range from 100 to 260° or its equivalent on the Fahrenheit scale

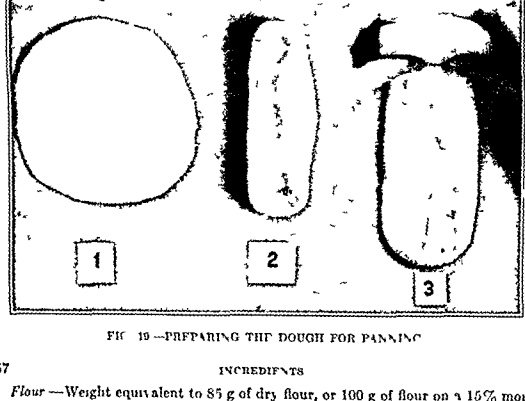

FIG 19—PREPARING THE DOUGH FOR PANNING

57 INGREDIENTS

Flour—Weight equivalent to 85 g of dry flour, or 100 g of flour on a 15% moisture basis

Yeast—3 g

Salt—1 g

Sugar (sucrose)—2 5 g

Water (distilled)—Sufficient to make 58% absorption with the flour on a 15% moisture basis To determine the exact quantity of H_2O to be added subtract from 178 the weight of flour used as previously computed

58 PROCEDURE

Mixing—Dissolve the salt and sugar in a portion of the H_2O Disintegrate the weighed portion of yeast in the salt sugar soln or in another portion of the H_2O If the former procedure is followed do not allow the yeast to remain in the salt

sugar soln for any considerable time before adding to the flour If stock solns are used, correct for the H_2O added Add the flour and mix with a flexible spatula that will conform readily to the shape of the bowl, making 125 cuts with the spatula So regulate the temp of the ingredients that the dough comes from the mixing operation at 30° Remove the dough from the bowl and fold 20 times in the hands

Fermentation—Place the dough in a fermentation bowl and allow to ferment for 105 min at 30° (plus or minus 0 5°) and at not less than 75% relative humidity Remove the dough from the bowl, fold 15 times in the hands (first punch), return the dough to the bowl, and allow fermentation to proceed as before for 50 min Again remove the dough, fold 10 times (second punch), and replace in the fermentation bowl for 25 min Remove the dough from the bowl, mold, and pan as follows

Molding and panning—Place the dough on a table or molding board and pound vigorously with the heel of the hand until the dough is flat and circular (Fig 1) Holding one side of the dough, cut the mass loose from the table with the spatula and turn on the reverse side Fold over two opposite sides so that they overlap to a considerable degree (Fig 2) Turn the dough over, and again pound it flat with the heel of the hand Holding one end, cut the dough loose from the table with a spatula, and turn on reverse side with the seam of the dough running from the operator Starting at the more remote end, roll the dough toward the operator, folding it as tightly as possible (Fig 3) Seal the seam tightly, and with the seam underneath seal the ends by pinching them vertically Roll lightly under the palm of the hand adjusting the dough to the length of the pan, and place in the pan with the seam down (The length of the dough prior to the final light rolling should not exceed that of the pan Use no dusting flour in the molding process

Proof—Proof 55 min under the same conditions as for fermentation

Baking—Bake 25 min at a temp of 230 (plus or minus 5°) at the level of the top of the baking pan (Precise control of temp both as to degree and uniformity, is essential)

Measurement—Weigh the loaf and measure its volume 30 min after removal from the oven

59 *Optional Variations*

1 *Absorption* Basic procedure in which absorption only is varied

2 *Fermentation* Basic procedure varying fermentation time only

3 *Addition of special oxidizing reagent $KBrO_3$* Basic procedure with addition of $KBrO_3$ in successive increments of 1 mg Prepare a soln of $KBrO_3$ so that 1 cc = 1 mg of $KBrO_3$ using $KBrO_3$ of highest purity

4 *Method of mixing* Basic procedure varying mixing by use of mechanical mixer

ALIMENTARY PASTES

60 COLLECTION AND PREPARATION OF SAMPLES—TENTATIVE

Select from the lot to be analyzed sufficient strips or pieces to assure a representative sample Break these into small fragments with the hands or in a mill and mix well Grind 300–500 g in a mill until all the material just passes thru a 20 mesh sieve Keep the ground sample in a sealed container to prevent moisture changes

TOTAL SOLIDS AND MOISTURE —OFFICIAL FIRST ACTION

61 *I Indirect Method*[26]

Determine the total solids in the sample prepared as directed under 60, as directed under 3

62 *II Air Oven Method*

Proceed as directed under 5, using a sample prepared as directed under 60

63 ASH—OFFICIAL

Proceed as directed under 6, using 3–5 g of the sample prepared as directed under 60

64 CHLORIDES IN ASH AS SODIUM CHLORIDE—OFFICIAL

Dissolve the ash obtained under 63 in HNO_3 (1+9), filter, wash the filter paper with hot H_2O, and determine Cl in the combined filtrate and washings as directed under XII, 33 or 35 Calculate the Cl to its equivalent of NaCl

65 FAT (ACID HYDROLYSIS METHOD)[a]—OFFICIAL, FIRST ACTION

Place 2 g of the sample in a Rohrig or Mojonnier fat extraction tube add 2 cc of 95% (by volume) alcohol, and shake so as to moisten all particles (The moistening of the sample with alcohol prevents lumping on addition of the acid) Add 10 cc of HCl (25+11) mix well set the tube in a water bath held at 70–80°, and shake at frequent intervals for 30 40 min Fill to within 1–2 cc of the mark with 95% alcohol and cool Add 25 cc of ethyl ether and shake the mixture well Then add 25 cc of redistilled petroleum ether (b p below 60°) and mix well Let stand until the upper liquid is practically clear and proceed as directed under 8, beginning "Draw off as much as possible

66 CRUDE FIBER—OFFICIAL FIRST ACTION

Proceed as directed under XXVII, 17–19

67 PROTEIN—OFFICIAL

(Organic and Ammoniacal Nitrogen)[a]

Determine the N as directed under II, 19, 22, or 24, using 1 g of the sample prepared as directed under 60 Multiply the percentage of organic and ammoniacal N by the factor 5 7 to obtain the percentage of protein

68 WATER SOLUBLE PROTEIN NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL—OFFICIAL FIRST ACTION

Proceed as directed under 25

69 LIPOID AND LIPOID PHOSPHORIC ACID (P_2O_5)—OFFICIAL FIRST ACTION

Proceed as directed under 26 and 27

70 UNSAPONIFIABLE RESIDUE—TENTATIVE

I I A C Method

Proceed as directed under 28, using 5 g of alimentary paste

71 *II Kerr Sorber Method*

Proceed as directed under 33

72 EXTRACTION AND IDENTIFICATION OF ADDED COLOR[a]—OFFICIAL

Transfer 50 g of the sample prepared as directed under 60 to a 200 cc nursing bottle, add about 125 cc of amyl alcohol, stopper, and shake well Add 27 cc of HCl (1+1) and agitate in a mechanical shaker until most of the color is extracted (15–30 min) Centrifugalize and pour off the amyl alcohol into a 250 cc separatory funnel Separate the colors in the amyl alcohol fractionally by successive washings with

HCl of decreasing concentrations, as 4 N, N, 0 25 N, etc , and then with H_2O until the washings are neutral (XXI, 7) (A roughly approximate 4 N acid is made by diluting 300 cc of HCl to 1 liter, and the other concentrations by using 75 cc, 19 cc, etc , of strong acid, respectively) Now add an equal volume of petroleum ether (b p below 60°) to the amyl alcohol and wash again with H_2O Finally wash the ether alcohol mixture with 10% NaOH soln Most of the egg and wheat colors as well as basic coal tar dyes and some others remain in the amyl alcohol ether mixture Identify the colors in the various fractions as directed under XXI, 12 and 19 Some colors are more or less colorless in acid soln and are apparent to the eye only after neutralization

73 DETECTION OF THE PRESENCE OF WHOLE EGG OR COMMERCIAL YOLK SOLIDS—TENTATIVE

Calculate the following ratios as percentages

(1) $\frac{100W}{N}$, (2) $\frac{100W}{L}$, and (3) $\frac{100P}{W}$, in which

W = the water soluble protein-N precipitable by 40% alcohol (24),
N = the organic and ammoniacal N (67)
I = the lipoids (26), and
P = the lipoid P_2O_5 (27)

Compare the values of these ratios with those obtained in the analysis of noodles of known composition [30]

74 EGG SOLIDS[31]—TENTATIVE

Calculate the percentage of egg solids in the sample from the lipoid P_2O_5 content by means of the following basic values and formulas

(a) Basic values

0 055% = lipoid P_2O_5 of flours, average value (dry basis),
1 38 % = lipoid P_2O_5 of whole eggs, average value (dry basis), and
1 78 % = lipoid P O_5 of commercial yolk, average value (dry basis)

(The above basic values are from a limited number of analyses, but it is extremely unlikely that more extended investigations will alter them materially)

(b) Formula for percentage of whole egg solids

$E = \frac{(A - 0\ 055)\ 100}{1\ 38 - 0\ 055}$ or $(A - 0\ 055)\ 75\ 5$, in which

E = percentage of whole egg solids in sample (dry basis), and
A = percentage of lipoid P_2O_5 in sample (dry basis) times 1 1, to correct for loss of lipoid P_2O_5 in manufacturing process [31]

Then

Whole egg solids in original sample =

$$\frac{E \times \text{percentage of dry matter in noodles}}{100}$$

(c) Formula for percentage of commercial egg yolk

For samples made with commercial egg yolk use the following formula

$Y = (A - 0\ 055)\ 58\ 0$ in which

Y = percentage of commercial yolk solids in sample (dry basis), and
A = percentage of lipoid P O_5 in sample (dry basis) times 1 1 to correct for loss of lipoid P O_5 in manufacturing process [31]

Then

$$\text{Commercial yolk solids in original sample} = \frac{\text{I} \times \text{percentage of dry matter of noodles}}{100}$$

SELECTED REFERENCES

[1] J Assoc Official Agr Chem 9, 39 (1926)
[2] Ibid, 8, 661 (1925), 9, 39 (1926)
[3] Ibid, 8, 665 (1925), 9, 39 (1926)
[4] Ibid, 7, 132 (1923)
[5] Ibid, 6, 505 (1922), 9, 41 (1926)
[6] Ibid, 10, 33 (1927), 11, 37 (1928)
[7] U S Dept Agr Bur Chem Bull, 152, p 101
[8] Ibid, 122, p 51
[9] J Assoc Official Agr Chem, 12, 39 (1929)
[10] Ann Phys Chem, 85, 161 (1852) U S Dept Agr Bur Chem Bull 122, p 127
[11] J Assoc Official Agr Chem 7, 81 (1924) 12, 39 (1929), 14, (1931)
[12] Ibid, 7, 91 (1923), 9, 40 (1926)
[13] Ibid, 7, 91 (1923), 9, 41 (1926)
[14] Ibid, 9, 45 (1926) 10, 35 (1927), 11, 37 (1928)
[15] Ibid, 8, 111 (1925) 10, 33 (1927)
[16] Ibid, 10, 108 (1927), 11, 37 (1928)
[17] Ibid, 6, 68 (1922), 7, 130 (1923)
[18] Ibid, 11, 134 (1928)
[19] Ibid, 9, 32 (1926)
[20] Ibid 8, 585 (1925) 9, 42 (1926)
[21] Ibid, 9, 42 (1926)
[22] Ibid, 6, 61 (1922)
[23] Ibid, 12, 41 (1929)
[24] Cereal Chem, 5, 220 (1928)
[25] J Assoc Official Agr Chem, 9, 43, 396 (1926)
[26] Ibid 9, 43, 397 (1926)
[27] Ibid, 11, 38 (1928) 6, 508 (1923)
[28] Ibid, 9, 43 (1926)
[29] Ibid 6, 12 (1922) 8, 109(1924), U S Dept Agr Bull 448
[30] J Assoc Official Agr Chem, 7, 84 (1923) 407 (1924) 8, 435 (1925)
[31] Ibid, 7, 91 (1923)

XXI COLORING MATTERS IN FOODS—TENTATIVE

(The numbers in parentheses and brackets following the name of a dye represent in the first instance the number of that dye as listed in "A Systematic Survey of the Organic Colouring Matters," founded on the German of Drs G Schultz and P Julius, 1904, by Arthur G Green, while the second number designates the number as listed in the Society of Dyers and Colourists "Colour Index," first edition, January, 1924)

1 PIGMENTS

Separate the insoluble pigments, ultramarine, lampblack, etc , which are most commonly used as facings, by washing the sample with H_2O and allowing the washings to settle Identify the particles of coloring matter by microscopical examination and treat the residue or purified coloring matter with chemical reagents A large proportion of the common pigments other than lakes, such as the yellow, brown, and red ochres and umbers, are derivatives of the heavy metals and contain Fe, Mn, etc Others, such as the green and blue compounds, including certain green chlorophyll derivatives, may contain Cu These pigments may be identified by the usual tests for the respective metals The analytical properties of the insoluble coloring matters are described in various standard works, some of which are listed under the selected references, especially Farbstofftabellen by Schultz[1] and Colour Index

SOLUBLE COLORING MATTERS AND THEIR LAKES

WOOL DYEING PROCEDURE[2]

2 *I Water-soluble coal tar dyes*

(a) *Wines fruit juice , distilled liquors, flavoring extracts, vinegars, beers, sirups, non alcoholic beverages, and similar products* —Dilute 20–200 cc of the sample with 1–3 volumes of H_2O, neutralize with NH_4OH (1+9) if necessary, and boil or heat on a steam bath with a small piece of white woolen cloth (nun's veiling) If the mixture contains much alcohol, heat until most of it has been removed, in other cases take out the wool after 5–15 min and rinse with H_2O Then treat the liquid with 3 or 4 drops of HCl for each 100 cc of soln and warm again for 10–20 min with a clean piece of wool If the wool takes up much coloring matter in either case, the presence of coal tar dyes is indicated

The basic colors dye the fiber best from neutral or faintly ammoniacal solns and, if present, they will appear on the first piece of wool Acid colors dye from neutral solns, but more readily from those containing free acid The lichen colors[3] (archil, cudbear, litmus) go readily on wool however and many other natural colors such as turmeric, will dye the fiber if present in considerable amount On the other hand a few coal tar dyes, especially auramine O and naphthol green B, are quite unstable, and if present in small quantities may give no distinct dyeing Acid dyes are much more frequently used than basic dyes, and in most cases they may be removed from wool without much decomposition by "stripping" the latter with dilute NH_4OH[4] Many natural colors are destroyed by the action of the alkali, while others remain for the most part on the fiber

If the behavior with wool in neutral and acid solns indicates the presence of acid dyes, rinse the colored cloth thoroly with H_2O cover with NH_4OH (1+9) in a casserole and boil for a few min Remove the cloth and squeeze out the adhering liquid Boil the ammoniacal soln to remove the excess of NH_3, drop in a piece of

clean wet wool, make distinctly but not strongly acid with HCl (1+9), and boil again If acid coal tar dyes are present, they will usually give a fairly clean, bright dyeing on the second piece of wool A further purification may be carried out by repeating the stripping and redyeing, though this procedure is generally accompanied by a corresponding loss of dye

(b) *Candies and similar colored sugar products* —Dissolve about 20 g of the sample in 100 cc of H_2O and treat the soln as directed under (a) When the coloring matter is on the surface of the candy pour off the soln before the colorless inner portion has dissolved

(c) *Jams and jellies* —Boil a mixture of 10–20 g of the sample and 100 cc of H_2O with wool in neutral and also in acid soln as directed under (a) For thick jams it is usually better, though less easy first to extract the coloring substances by treating the product as directed under (d)

(d) *Canned and preserved fruits and vegetables, sausage casings, smoked fish, coffee, spices, etc* —Macerate 20–200 g of the sample with 4–5 times its weight of alcohol, 80% by volume Allow to stand a few hours, pour off the solvent as completely as possible and repeat the extraction using alcohol 70% by volume and containing approximately 1% of NH_4OH (1) Examine separately the filtered alcoholic extracts as directed under (a), or, (2) boil the ammoniacal soln until practically neutral complete the neutralization with acetic acid, add the neutral 80% alcohol extract, continue the evaporation until most of the alcohol is removed and boil a small portion with wool as directed under (a)

(e) *Cocoa and chocolate products* —Treat cocoa as directed under (d) The alcoholic extract will contain large quantities of natural coloring matters, and several dyeings and strippings may be necessary to remove these in order to show the presence of coal tar dyes

Chocolate may be treated similarly, but the following procedure is preferable Wash 20–200 g of the well divided sample with gasoline on a filter until most of the fat has been removed, if the gasoline is colored reserve for the examination of oil soluble dyes as directed under 3 Remove most of the adherent solvent from the residue by evaporation or pressure between layers of absorbent paper and digest with alcohol as directed under (d)

Coal tar dyes may also be detected in chocolate and cocoa products by mixing the samples directly with 3–4 times their weight of hot H O and immediately boiling the magma with wool as directed under (a)

(f) *Cereal products* —Proceed as directed under (d), in most cases working with a large quantity of the sample, 200–500 g, and a relatively smaller quantity of alcohol If tests are to be made for the acid dyes only the extraction with neutral 80% alcohol may be omitted advantageously In the case of macaroni products, proceed as directed under XX, 72

3 *II Oil soluble coal tar dyes*[3]

Prepare an alcoholic soln of the dye by applying one of the following methods to the oil or fat obtained by extraction with ether or gasoline if the nature of the substance requires it

(a) Shake the oil or melted fat with an equal volume of alcohol, 90% by volume and wash the alcoholic extract with several portions of gasoline to free the coloring matter from foreign fats The alcohol, after separation, will contain aniline yellow butter yellow, aminoazotoluene auramine etc if present

(b) Saponify 20–200 g of the oil or fat with 0 5 *N* alcoholic KOH, remove most of the alcohol on the steam bath, and extract the soap with ether or gasoline Remove the dyes from the solvent with 10 cc portions of HCl (1+3) Most of the common dyes are removed by this treatment, tho the digestion with strong alkali may cause some decomposition and make the extraction rather troublesome

(c) Dilute 20–200 g of the oil or melted fat with 1–2 volumes of gasoline and shake out successively with 2–4% KOH or NaOH soln, HCl (1+3), and H_3PO_4–H_2SO_4 mixture, prepared by mixing 85% H_3PO_4 with about 10–20% by volume of H_2SO_4 The dilute alkali extracts sudan G (10) [23] and annatto (709) [1241] The dilute HCl extracts aniline yellow (7) [15], aminoazotoluene (—) [17], and butter yellow (16) [19], the first two forming orange red the latter cherry-red solns in this solvent The H_3PO_4 mixture is necessary for the extraction of sudan I (11) [24], sudan II (49) [73], sudan III (143) [248], and the homologue of the last, sudan IV (—) [258] Benzeneazo beta naphthylamine (—) [22] and homologues also come in in this group, tho they readily undergo chemical changes in the strongly acid mixtures The procedure is not very suitable in the presence of auramine, but this dye is seldom found in oils Neutralize the alkaline and the dilute HCl solns dilute the H_3PO_4 mixture and partially neutralize, cooling the liquid during this operation, and extract the dyes by shaking with ether or gasoline

For the direct dyeing test use the alcoholic soln obtained as directed under (a) Evaporate to dryness the ether or gasoline solns obtained as directed under (b) and (c) and dissolve the residue in 10–20 cc of 95% alcohol To the alcoholic soln add some strands of white silk and a little H_2O and evaporate on a steam bath until the alcohol has been removed or the dye is taken up by the silk The dyeing test is sometimes unsatisfactory and in all cases a small portion of the alcoholic soln should be tested by treating with an equal volume of HCl and $SnCl_2$ soln The common oil soluble coal tar dyes are rendered more red or blue by the acid and are decolorized by the reducing agent Most of the natural coloring matters become slightly paler with the acid and are little changed by the $SnCl_2$ soln

SEPARATION OF COLORING MATTERS IN PURE CONDITION BY MEANS OF IMMISCIBLE SOLVENTS[3]

4 *Coal Tar Dyes in General*

The use of immiscible solvents for the separation of mixtures of coloring matters usually requires a systematic fractionation since many dyes do not differ very greatly in their solubilities in the various solvents

5 PREPARATION OF SOLUTION

(a) *Water-soluble colors* —Proceed as directed under 2, omitting the fixation of the color on wool, and obtain an aqueous soln as free as practicable from suspended matter, alcohol, acids, alkalies, and salts Liquids require no preparation except the removal of any alcohol that may be present

(b) *Water insoluble lakes* —If the sample is in solid form, treat the well divided material with sufficient H_2O to form a paste

(c) *Oil soluble dyes* —Proceed as directed under 3, preferably 3 (a) or 3 (c)

The dye concentration should lie preferably between 0 01 and 0 05% The soln obtained in the examination of colored food products rarely requires further dilution, but with commercial food colors the concentration should not be too great

6 *Basic Dyes*

Most basic dyes may be separated from mixtures by making alkaline with 10% NaOH soln and shaking with ether[7] Use the sample, prepared as directed under 5,

for this purpose Separate the ether layer, which may or may not be colored, wash it twice with a few cc of H_2O to remove excess of alkali, and shake with acetic acid (1+18), which will take up any dye present and form a colored soln Altho this treatment may, to some extent, alter the common basic colors, it can be used for the detection of methyl violet B (451) [680], magenta (448) [677], bismarck brown (197) [331], malachite green (427) [657], and rhodamine B (504) [749] With care auramine (425) [655] also may be separated in this way, tho it is quickly decomposed on standing in alkaline soln

Acid Dyes

7 The following short procedure is often convenient for the examination of mixtures of acid dyes Make the sample prepared as directed under 5, strongly acid by adding ½ its volume of HCl and shake with amyl alcohol Separate the amyl alcohol soln and wash by shaking with successive portions of ¼ its volume of H_2O, reserving the portions in separate test tubes or beakers Because of the varying acid content of the amyl alcohol these washings will show a regular decrease in acidity and the coloring matters will appear in maximum quantity in the different fractions according to their respective solubilities Ponceau 6R (108) [186] is washed out chiefly while the acidity is still high, normal or above Amaranth (107) [184], brilliant scarlet (106) [185] and tartrazine (94) [640] appear when the washings have an acidity between normal and 0 25 *N*, orange G (14) [27] and soluble blue (480) [707] between 0 5 *N* and ⅛ *N*, and palatine scarlet (53) [77] ponceau 2R (59) [79] and 3R (56) [80], naphthol yellow S (4) [10] cochineal (706) [1230] crystal ponceau (64) [89], and azorubine A (103) [179] between ⅛ *N* and 1/256 *N* Even practically all the acid is removed, orange I (85) [150], orange II (86) [151] and croceine orange (13) [26] begin to wash out, and less readily orange IV (88) [143] and metanil yellow (95) [138] Finally the unsulfonated coloring matters, such as erythrosine G (516) [772] erythrosine B (517) [773], and the rose bengals (520) [777] and (523) [779] are removed very slowly by H_2O or not at all unless the solvent is diluted with gasoline and the dyes are removed with H_2O containing a few drops of NH_4OH Acid yellow (8) [16] and brilliant yellow S (89) [144] are not very uniform in composition They are partially taken up by amyl alcohol from acid soln and appear chiefly in the first washings Indigotine (692) [1180] behaves somewhat similarly

When it appears probable that only the coal tar dyes listed in the regulations for the enforcement of the Federal Food and Drugs Act[8] for use in food products are present the following abridged procedure may be conveniently used for their separation

PERMITTED COAL TAR FOOD COLORS[8]

8 (*Amaranth ponceau 3R erythrosine, orange I, light green SF yellowish fast green FCF, guinea green B, indigotine naphthol yellow S tartrazine yellow 4B, and yellow OB*) No methods have been adopted for brilliant blue, ponceau 5R, and sunset yellow

PREPARATION OF SOLUTION

(a) *For foodstuffs containing oil soluble dyes*—Proceed as directed under 3 (a), evaporate the 90% alcoholic extract to dryness in a casserole, treat the residue with 40 cc of low boiling gasoline and shake the gasoline soln with 2 or 3 portions of 5 cc each of 2–4% NaOH soln (to remove annatto, turmeric, etc, if present) The gasoline soln will contain the yellow OB and yellow AB

(b) *For foodstuffs which contain no oil soluble dyes or from which these dyes have been removed*—Proceed as directed under 2, omitting the fixation of the color on wool and

obtain an aqueous soln as free as possible from suspended matter, alcohol, acids, alkalies, and salts The dye soln should be preferably between 0 01 and 0 05% The soln obtained in the examination of colored food products rarely requires further dilution, but with commercial food colors care must be taken that the concentration is not too great

9 SEPARATION

(a) *Yellow AB and yellow OB* —Extract the gasoline soln of these dyes, as obtained under 8 (a), 3 times with ½ its volume of 13 *N* H_2SO_4 Shake each acid extract successively with 2 portions (equal volumes) of low boiling gasoline, using the same 2 portions of gasoline for each acid portion Extract each of the 2 latter gasoline portions with 20 cc of 13 *N* H_2SO_4, using the same acid portion successively for both gasoline portions Finally extract the second of these gasoline portions with another 20 cc portion of 13 *N* H_2SO_4 (The original gasoline soln has now been shaken with acid 3 times, the next gasoline portion 4 times and the third 5 times) Combine the acid extracts dilute with water, re extract with low boiling gasoline, and evaporate the solvent Yellow AB will be found in a practically pure state Combine the gasoline solns(original and subsequent solns left after the acid washings), wash with small portions of H_2O to remove excess of acid, and evaporate the solvent The yellow OB will remain as a residue (This method is not absolutely quantitative, but it is sufficiently accurate to make a separation of either of the dyes with comparatively little contamination from the other) The following color test may be applied to the separated dyes to confirm their identity Shake 5 cc of a neutral gasoline soln of the dye in a test tube with 5 cc of a mixture of 1 part of 40% HCHO soln and 4 parts of acetic anhydride Both coloring matters are extracted by the acetic anhydride, yellow AB giving in a few seconds a red colored soln and yellow OB, under the same conditions, giving an orange colored soln

(b) *Amaranth ponceau 3R, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, indigotine, naphthol yellow S, and tartrazine* —To the soln obtained under 8 (b), add sufficient 25% salt soln to make the concentration about 10% and 1 part acetic acid to every 7 parts of soln Extract with 3–50 cc portions of amyl alcohol Draw off the lower layer and reserve for further treatment Wash the amyl alcohol extract in rotation with 25 cc portions of 5% salt soln until the washings are colorless or nearly so Add the washings to the original aqueous soln Dilute the amyl alcohol extract with equal volume of gasoline and wash with 25 cc portions of H O until all color is extracted The coloring matters obtained are orange I and guinea green B For their separation see (1) below Treat the amyl alcohol gasoline soln with 10 cc portions 0 1 *N* NaOH or with 10 cc portions of NH_4OH (1+9), which will remove erythrosine The original soln and washings (from which the 3 named dyes were removed) are acidified with HCl (1 volume acid to 40 volumes of soln) and extracted in 50 cc volumes with 3–50 cc portions of amyl alcohol Reserve the lower aqueous layer for further treatment Wash amyl alcohol extract with 25 cc portions of 0 25 *N* HCl until the washings are colorless or nearly so Combine washings with the aqueous soln of above The amyl alcohol is extracted with several 25 cc portions of H_2O until all color is extracted The coloring matters obtained are ponceau 3R and naphthol yellow S For their separation see (2) The original soln and washings (from which the 5 named dyes were removed) are treated in 50 cc volume with 3–50 cc portions of dichlorhydrin Reserve the upper aqueous layer for further treatment Wash the dichlorhydrin extract in rotation with several 20 cc portions of 25% salt soln Combine washings with the aqueous soln of

above The dichlorhydrin extract is diluted with 2 volumes of CCl_4 and extracted with several 25 portions of H_2O until all color is extracted The coloring matters ob tained are light green SF yellowish and fast green FCF For their separation see (3) Further acidify the original soln and washings (from which the 7 named dyes were removed) with HCl (1 volume of acid to 40 volumes of soln) and extract in 50 cc volumes with 3–50 cc portions of amyl alcohol If the color intensity of the soln was not too strong, all coloring matter should have been extracted by the solvent Discard the lower colorless or nearly colorless layer and wash out the dyes from the amyl alcohol extract in rotation with several 25 cc portions of H O, until all color is extracted The coloring matters obtained are indigotine, amaranth and tartrazine For their separation see (4)

(1) *Orange I and guinea green B* —Extract the combined colors with 2–20 cc portions of α dichlorhydrin Discard the colorless upper aqueous layer dilute the sol vent with 2 volumes of CCl_4, and extract out orange I in rotation with several 10 cc portions of H_2O, and guinea green B with several 10 cc portions of 25% alcohol

(2) *Ponceau 3R and naphthol yellow S* —Acidify the combined colors with HCl (1 part acid to 10 parts of soln) and extract the naphthol yellow S with 2–20 cc por tions of washed ethyl acetate or amyl acetate Ponceau 3R is not extracted appre ciably and remains in the aqueous layer Wash the solvent with 5 cc portions of *N* HCl to remove traces of ponceau 3R Naphthol Yellow S is removed from the com bined ethyl acetate or amyl acetate with 5 cc portions of NH_4OH (1+9)

(3) *Light green SF yellowish, and fast green FCF* —The combined colors are treated with an equal volume of 2 *N* Na_2CO_3 soln and extracted in 25 cc volumes with 2–50 cc portions of *N* butyl alcohol Draw off the lower aqueous layer contain ing the fast green FCF and remove the last traces with 25 cc portions of *N* Na_2CO_3 until the washings are colorless The light green SF yellowish can be removed from the butyl alcohol with 10 cc portions of 1% acetic acid

(4) *Indigotine amaranth and tartrazine* —to separate the indigotine heat a small portion of the soln which should be neutral or faintly acid, to boiling, and add a few crystals of $NaHSO_3$ until all the dyes are reduced On shaking with air the in digotine is quickly restored, while amaranth and tartrazine are destroyed To the remainder of the mixed dye soln add several decigrams of urea, heat, and while the mixture is boiling add 1 or 2 drops of 10% $NaNO_2$ Indigotine is converted to the pale yellow isatin sulfonate while amaranth and tartrazine are but little affected The resultant soln is acidified with HCl, and the dyes are extracted with 2–20 cc portions of amyl alcohol, the isatin compound being less readily extracted by the solvent To remove amaranth and tartrazine shake out the amyl alcohol extract with 10 cc portions of 0 25 *N* HCl Amaranth is much more readily attacked by re ducing agents than tartrazine in acid solns than in alkaline If an alkaline soln of the mixed dyes is carefully treated with a few crystals of NaHSO , amaranth will be destroyed while the tartrazine will be left practically unaltered If HCl is added to the mixture, and the dyes is extracted with small portions of amyl alcohol tar trazine can be separated in pure state with 0 25 *N* HCl

10 IDENTIFICATION[11]

The most widely used tests for the identification of coal tar dyes refer to the changes produced with acids and alkalies Other tests based upon the behavior with reducing agents followed perhaps by treatment with oxidants or by separation and identification of the reduction products [11] and tests based upon oxidation of the dye and treatment of the oxidation products,[12] are generally applicable Spectroscopic methods are also used [13]

TABLE I

Color reactions produced on dyed fibers by various reagents

COLORING MATTER	C I NO	S & J NO	STRONG HYDROCHLORIC ACID	CONCENTRATED SULFURIC ACID	10% SODIUM HYDROXIDE SOLUTION	DILUTE AMMONIUM HYDROXIDE
Rhodamine B	*749*	504	Orange	Yellow	Bluer	Bluer
Rose Bengal	*779*	523	Almost decolorized	Orange	No change	No change
Archil	*1242*	710	Red	Reddish brown	Violet	Violet
Magenta	*677*	448	Yellowish brown	Yellowish brown	Decolorized	Paler
Acid Magenta	*692*	462	Almost decolorized	Yellow	Decolorized	Decolorized
Palatine Red	*85*	62	Darker	Blue	Dull brown	Little change
Bordeaux B	*88*	65	Violet	Blue	Brick red	Little change
Amaranth	*184*	107	Slightly darker	Violet to brownish	Dull brownish to orange red	Little change
						Little change
Azorubine A	*179*	103	Little change	Violet	Red	Red
Erythrosine	*773*	517	Orange yellow	Orange yellow	No change	No change
Ponceau 6RB	*286*	169	Blue	Blue	Dull violet red	Little change
Ponceau 6R	*186*	108	Violet red	Violet	Brown	Orange red
Crystal Ponceau	*89*	64	Red	Violet	Dull brown	Little change
Ponceau 3R	*80*	56	Little change	Little change	Dull orange	Little change
Sudan III*	*248*	143	Violet, then brown	Green	Violet red	Little change
Safranine	*841*	584	Greenish blue	Green	Red	Red
Brilliant Scarlet	*185*	106	Red	Violet-red	Yellowish brown	Orange-red
Ponceau 2R	*79*	55	Little change	Little change	Brownish yellow	No change
Palatine Scarlet	*77*	53	Darker	Violet red	Brownish yellow	No change
Erythrosine G	*772*	516	Yellow orange	Yellow orange	No change	No change
Sudan II*	*78*	49	Red	Violet red	Little change	No change
Sudan I*	*24*	11	Orange red	Red	Redder	No change
Cochineal	*1239*	706	Little change	Little change	Violet red	Violet red
Bismarck Brown	*331*	197	Redder, darker	Browner	Yellower	Yellower
Bismarck Brown R	*332*	201	Redder, darker	Browner	Yellower	Yellower
Orange I	*150*	85	Violet	Violet	Red dark	Red, dark
Orange II	*151*	86	Red	Red	Dull red	No change
Croceine Orange	*26*	13	Orange red	Orange	Slightly darker	No change
Orange G	*27*	14	Little change	Orange	Dull, brownish red	No change
Orthotolueneazobeta naphthylamine* (Yellow OB)	*61*		Red	Violet	Little change	No change

* Oil soluble

Benzeneazobeta-naphthylamine* (Yellow AB)	22		Red	Violet	Little change	No change
Sudan G*	23	10	Orange yellow	Brownish yellow	Orange yellow	No change
Butter Yellow*	19	16	Violet red	Orange yellow	No change	No change
Aniline Yellow*	15	7	Violet red	Orange yellow	Little change	No change
Aminoazoorthotoluene*	17		Dull orange	Orange-yellow	Little change	No change
Fluorescein	766	510	Little change	Little change	Green fluorescent	Green fluorescent
Metanil Yellow	158	95	Violet red	Violet	No change	No change
Azoflavine	145	92	Violet red	Violet red	Dull brown	Little change
Acid Yellow	16	8	Red	Orange	Little change	No change
Brilliant Yellow S	144	89	Violet red	Violet red	Little change	Little change
Tartrazine	640	94	Slightly darker	Slightly darker	Little change	Little change
Naphthol Yellow S	10	4	Almost decolorized	Very pale dull brown	No change	No change
Auramine	655	425	Decolorized	Almost decolorized	Decolorized	Paler
Turmeric	1258	707	Red	Reddish brown	Orange	Orange
Quinoline Yellow	801	667	Slightly darker	Brownish yellow	Slightly paler	Little change
Naphthol Green B	6	393	Yellowish	Brownish yellow	No change	No change
Guinea Green B	666	433	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Light Green SF Yellowish	670	435	Pale orange yellow	Yellowish brown	Decolorized	Decolorized
Fast Green FCF			Orange	Green to brown	Blue	Blue
Night Green 2B	687	438	Pale orange yellow	Yellowish brown	Decolorized	Paler
Malachite Green	657	427	Almost decolorized	Almost decolorized	Decolorized	Decolorized
Erioglaucine A	671	436	Yellow	Pale dull yellow or brown	Slightly darker	Little change
Patent Blue A	713	442	Pale orange yellow	Green to brown	Little change	Little change
Soluble Blue	707	480	Paler	Brown	Pale reddish	Almost decolorized
Indigotine	1180	692	Slightly darker	Darker	Greenish yellow	Greenish blue
Formyl Violet	698	468	Pale orange-yellow	Pale dull orange	Decolorized	Decolorized
Methyl Violet	680	451	Yellowish	Yellowish	Decolorized	Almost decolorized
Nigrosine, soluble	865	602	Dull bluish	Dull greenish	Brownish red paler	Pale reddish

I By Color Changes Produced with Acids and Alkalies

11 REAGENTS

(a) *Hydrochloric acid*
(b) *Sulfuric acid*
(c) *Sodium hydroxide soln* —Dissolve 10 g of NaOH in H_2O and dilute to 100 cc
(d) *Ammonium hydroxide* —Contains 12% NH_3 by weight

12 DETERMINATION

Transfer the separated coloring matter to wool (or to silk in the case of oil soluble dyes) by boiling as directed under 2 (a) or 3 Care should be taken that the final dyeing is made in a soln fairly free from foreign matter such as sugar or aromatic substances, which, adhering to the fiber, may modify the reaction In most cases the quantity of color available is small and should not be used to dye too large a piece of wool (or silk) Rinse the dyed fiber thoroly in running H_2O, dry, cut into small pieces, and place separately in the depressions of a white porcelain spot plate Moisten the pieces with the reagents described under 11 (For many coloring matters the hue upon treatment with acids or alkalies varies markedly with the concentration of the reagents and quantity of dye present, therefore the unknown dye should be compared with dyeings of known colors of approximately the same dye concentration as shown by their appearance)

The table under 13 shows the color changes produced on wool dyed with 0 1–0 5% solns of the respective coloring matters Included also are the reactions of the oil soluble colors, but these refer to dyeing on silk The dyes are arranged approximately according to hue Brown is classed with orange, black (gray), with violet

14 *II By Special Tests* [8]

(a) *Oil soluble dyes (yellow AB and yellow OB)* —A method of separating and identifying the 2 permitted oil soluble dyes is given under 9 (a) The alcoholic solns of these dyes become red on treatment with HCl, are unaffected by alkalies, are reduced by $SnCl_2$ $TiCl_3$, and $NaHSO_2$, and the color is not restored to the reduced solns on the addition of $FeCl_3$ or K persulfate

(b) *Water soluble dyes (amaranth, ponceau 3R, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, indigotine, naphthol yellow S, and tartrazine)* —Treatment of these dyes in acid soln with SnCl , $TiCl_3$, Zn dust, or $NaHSO_2$ decolorizes indigotine, amaranth, tartrazine ponceau 3R, and orange I With indigotine the color returns on shaking with air, but more readily on warming or on the addition of $FeCl_3$ or K persulfate Excess of the reducing agents must be avoided With the last 4 named dyes the color is not restored Dilute solns of light green SF yellowish, guinea green B naphthol yellow S, and erythrosine become paler or colorless with acid so that the effects of acid reducing agents are not so readily apparent Neutral solns of naphthol yellow S are first changed to pink and later decolorized by NaHSO and other reducing agents the color not returning with air or oxidants Erythrosine, light green SF yellowish, fast green FCF, and guinea green B become paler with $NaHSO_2$ the color being partially restored upon the addition of K persulfate

In hot solns containing an excess of Na tartrate, the water soluble dyes are readily decolorized by $TiCl_3$ [14] In the case of indigotine, if the reducing agent has been added carefully and an excess avoided, the blue color readily returns on shaking with air With erythrosine, light green SF yellowish, fast green FCF, and guinea green B the color is scarcely restored by air, but on cooling and adding K-persulfate it re

turns imperfectly The reduction products of the other dyes do not give colored solns again on oxidation, if a slight yellowish or brownish tint that may sometimes appear is disregarded

(1) *Light green SF yellowish fast green FCF, and guinea green B* belong to the triphenyl methane type of dyes Solns of light green SF yellowish and guinea green B behave similarly with acids alkalies and reducing agents, producing a yellow to a greenish yellow with mineral acids, and an almost colorless solution with alkalies as well as with reducing agents On the other hand, while the reactions of fast green FCF are similar with acids and reducing agents they differ by producing a deep blue soln with NH_4OH and fixed alkalies as well as with a 10% soln of Ba acetate The easy solubility of these 3 greens in a dichlorhydrin differentiates them from all other permitted dyes To separate guinea green B from light green SF yellowish and fast green FCF, proceed as follows

Light green SF yellowish and guinea green B —Prepare a soln of 250 g of NaCl 27 g of crystallized Na acetate, and 24 cc of acetic acid in H_2O and dilute to 1 liter To separate and differentiate the 2 green coloring matters add to every 20 cc of dye soln 1 cc of HCl and extract with an equal volume of amyl alcohol Draw off the lower layer and remove the light green SF yellowish by washing the remaining amyl alcohol portion with equal volumes of the NaCl sodium acetate soln until no more color is extracted Dilute the amyl alcohol with an equal volume of gasoline and remove the guinea green B with water

Light green SF yellowish and fast green FCF —To separate and differentiate proceed as under 9 (3)

(2) *Indigotine* is extracted in small proportions from slightly acid solns by shaking with a dichlorhydrin from which it may be removed with small portions of 25% salt soln Most of the other common bluish dyes are triphenyl methane derivatives and are relatively more soluble in the solvent than in the aqueous layer Indigo is readily destroyed by boiling with a very small amount of a fixed alkali soln, by which treatment it may be readily eliminated from other coloring matters

(3) *Ponceau 3R* gives in neutral or faintly acid solns a bluish red flocculentprecipitate with $BaCl_2$ or Ba acetate practically all the dye being removed from soln Some of the soln obtained in the separation, under 9, may be used in this test, the free HCl first being neutralized with Na acetate, or better, it may be evaporated to dryness on a steam bath to remove the acid and the residue taken up with a little H_2O A brick red precipitate will be formed on standing when a neutral soln of the dye is treated with a 20% soln of neutral Pb acetate The soln should contain 0 005% or more of the dye

(4) *Naphthol yellow S*, in solns containing an excess of NH_4OH or Na_2CO_3, becomes intensely rose red on the addition of $NaHSO_3$, the color gradually fading again as complete reduction takes place, also a red coloration is produced if an aqueous soln of the dye is treated with a few drops of 40% $SnCl_2$ and an excess of 20% KOH is added

(5) *Tartrazine* is characterized by its comparative inactivity towards acids and alkalies, the solution of the dye being hardly altered by the reagents An alkaline soln of the dye is reduced with $NaHSO_3$ only with difficulty A concentrated neutral or slightly acid soln of the dye, when reduced with $SnCl_2$ soln or Zn dust and made slightly alkaline and filtered, will develop a purple coloration on standing

(6) *Orange I* can readily be recognized by its behavior to reagents With a large excess of HCl it produces a purplish red with alkali in large excess it produces a bright red soln

(7) *Erythrosine* differs from most of the common dyes in that it contains I To test for I, acidify the soln with H_2SO_4, shake with ether, separate the ether soln of the color, and evaporate to dryness in a Pt dish after the addition of a few drops of Na_2CO_3 soln or sufficient to form the deep red Na salt Hold the dish containing the residue in the Bunsen flame until organic matter is destroyed, take up the residue with H_2O, acidify with H_2SO_4, and test for I in one of the usual ways, such as with Cl water and CS or CCl_4, or with starch paste and an oxidizing agent It is useless to test for I with very small quantities of dye, but in most cases sufficient coloring matter can be separated from the food product to give satisfactory results

15 NATURAL COLORING MATTERS

As a class the natural coloring matters show much less tendency to dye animal fiber than do the common synthetic colors In many cases the crude products used contain a number of colored substances, and a complete separation is not practicable As dilute solns of most of the natural coloring matters are sensitive to alkalies and some are sensitive to acids, such reagents must be used with care Relatively few good tests are known for the common natural colors Some of their most useful analytical properties[15] are tabulated under 18

The properties of pure preparations of the various natural coloring matters are described, for the most part, by Rupe,[16] and by Perkin and Everest,[17] reference being made in these works to the original literature Properties of the chlorophylls and carotinoids are given by Willstätter and Stoll,[18] those of the coloring matters of the corn flower rose, pelargona flower, larkspur, cranberry, whortleberry, purple grape, sloe, cherry, plum, radish, and red beet are described by Willstätter and coworkers [19]

16 SEPARATION

(a) *By extraction with ether from neutral solns* —From neutral solns ether extracts carotin, xanthophyll (the pigments found in leaves, fats and oils, egg yolk, carrots, etc), the coloring matter of tomatoes and paprika, and green chlorophyll The coloring matter remains in the ether soln on shaking with normal NaOH soln or normal HCl, no apparent change taking place, altho chemically the substances may be altered more or less by this treatment

(b) *By extraction with ether from acid solns* —From slightly acid solns ether extracts very readily and completely the coloring matter of alkanet, annatto, turmeric, and the red dyewoods, sandalwood, camwood, and barwood It extracts in large proportions the flavone coloring matters of fustic, Persian berries and quercitron (after hydrolysis), as well as the coloring matter of Brazilwood and the green derivatives formed from chlorophyll by alkaline treatment It extracts in relatively small quantity the coloring matters of logwood, archil, saffron, and cochineal The coloring matters of this group are readily removed from ether by shaking with alkaline solns but in most cases they rapidly undergo chemical change

(c) *By extraction with amyl alcohol from acid solns* —From slightly acid solns amyl alcohol extracts largely the coloring matters of logwood, archil, saffron, and cochineal Amyl alcohol extracts in relatively small proportions caramel and the anthocyans constituting the red coloring matter of the most common fruits

IDENTIFICATION

I By color changes produced with various reagents

17 SOLUTIONS REQUIRED

(a) *Hydrochloric acid*

(b) *Sodium or potassium hydroxide soln* —10%

TABLE II

Reaction of certain natural coloring matters to common reagents

COLORING MATTER	STRONG HYDROCHLORIC ACID	10 PER CENT SODIUM HYDROXIDE SOLUTION	SODIUM HYPOSULFITE	0.5 PER CENT FERRIC CHLORIDE OLUTION	10 PER CENT ALUM SOLUTION	5 PER CENT URANIUM ACETATE SOLUTION	CONCENTRATED SULFURIC ACID ON DRY COLOR
Logwood	Deep red with excess of acid	Violet to violet blue	Almost decolorized color returning imperfectly by reoxidation	Dark shades of violet brown or black (the first hue often evanescent)	Rose-red (change rather slow)	Violet quickly fading	Red changing to yellow
Red woods (Brazilwood Sandalwood Camwood and Barwood)	Deep red with excess of acid	Violet red		Dark shades of violet brown or black (the first hue often evanescent)	Rose-red (change rather slow)		
Anthocyans of red fruit colors		Change to green dull blue or slate color usually very quickly becoming browner by oxidation	Anthocyanidins derived by hydrolysis almost completely decolorized				
Alkanet		Deep blue				Yellowish green	Violet blue
Archil	Little or no change	Blue	Decolorized color returning when shaken with air Reaction more easily seen in alkaline solution				Violet blue
Cochineal	Little or no change	Violet	No marked change	Slightly darker			Green
Annatto	Remains orange		Little affected	No marked change. Perhaps somewhat browner			Blue
Turmeric (solution in ether or alcohol characterized by pure yellow color and light green fluorescence)	Little change Orange-red or carmine-red on addition of several volumes of concentrated acid	Orange-brown	Little affected	No marked change Perhaps somewhat brown r	Little change	Somewhat browner	Red
Flavone colors of fustic Persian berries quercitron, etc	Becomes intensely yellow with volumes of concentrated acid	Bright yellow	Little affected	Olive-green or black colorations	More strongly yellow fustic developing a green fluorescence	Orange colorations	Yellow to orange
Saffron	Little or no change	Remains yellow	Little affected	No marked change. Perhaps somewhat browner	Little change	Not affected	Blue
Carotin and Xanthophyll	Little change Perhaps slightly paler	Little or no change	Little affected				Blue reaction obtained with difficulty
Green Chlorophyll	More brownish	Brown phase reaction, 20					
Caramel	Little or no change	Little change or slightly deeper brown	Slightly paler	No change			

(c) *Sodium hyposulfite* ($NaHSO$)

(d) *Ferric chloride soln* —0 5% Freshly prepared It may also be made by diluting a 10% stock soln

(e) *Potassium or ammonium alum soln* —10%

(f) *Uranium or sodium uranium acetate soln* —5%

(g) *Sulfuric acid*

19 DETERMINATION

Evaporate to dryness the ether solns obtained under 16 (a) and 16 (b), warm the residue with a little alcohol, and dilute with H_2O Dilute the amyl alcohol solns obtained under 16 (c) with H_2O To portions of these somewhat purified solns of the coloring matter apply the reagents in the following manner

Hydrochloric acid —Add the strong acid to the soln, first 1 or 2 drops, then a large excess equal to 3-4 times the volume of the soln

Sodium or potassium hydroxide —Make the soln slightly alkaline by adding a drop of the 10% NaOH or KOH soln

Sodium hyposulfite —Add a small crystal of the $NaHSO_2$

Ferric chloride —Add a small quantity of the 0 5% $FeCl_3$ soln to the soln to be tested very carefully, a small drop at a time, as the colorations are not obtained in some cases when an excess is used

Alum —Add to the test soln ½ its volume of the 10% K- or NH_4-alum soln

Uranium acetate —Add the 5% U-acetate soln dropwise to the soln to be tested

Sulfuric acid on the dry color —Evaporate a small quantity of the soln or of the coloring matter in a porcelain dish Cool thoroly and treat the dry residue with 1 or 2 drops of cold H_2SO_4 The colorations are in some cases extremely fugitive, and they may be observed only the instant the acid wets the residue

Table II, under 18, shows the behavior of certain of the natural coloring matters when treated in the manner described above

20 *II By Special Tests*

Chlorophyll —The "brown phase reaction"[20] may be useful for the characterization of chlorophyll, when this has not been previously treated with alkalies Treat the green ether or petroleum ether soln of the coloring matter with a small quantity of 10% soln of KOH in methyl alcohol The color becomes brown, quickly returning to green

21

Annatto[21] —Pour on a moistened filter an alkaline soln of the color obtained by shaking out the oil or melted and filtered fat with warm, dilute NaOH soln If annatto is present, the filter paper will absorb the color, so that when washed with a gentle stream of H_2O it will remain dyed a straw color Dry the filter and add a drop of $SnCl_2$ soln If the color turns pink, the presence of annatto is confirmed

22

Turmeric —Treat an aqueous or dilute alcoholic soln of the color with HCl until the shade just begins to appear slightly orange Divide the mixture into two parts and add some H_3BO_3 powder or crystals to one portion A marked reddening will be quickly apparent best seen by comparison with the portion to which the H_3BO_3 has not been added The test may also be made by dipping a piece of filter paper in the alcoholic soln of the coloring matter, drying at 100°, then moistening with a weak soln of H_3BO_3 to which a few drops of HCl have been added On drying again a cherry red color will be developed

23

Cochineal.—When the presence of cochineal is suspected acidify the mixture with ¼ its volume of HCl and shake with amyl alcohol Wash the amyl alcohol soln of the coloring matter 2–4 times with equal volumes of H_2O to remove HCl, etc Dilute the amyl alcohol with 1–2 volumes of gasoline and shake with a few small portions of H_2O to remove the color Divide the combined aqueous extracts into 2 portions To the first add, dropwise, 5% U-acetate soln, shaking thoroly after each addition In the presence of cochineal a characteristic emerald green color is produced [22] The green coloration with U salts is not developed in the presence of much free acid Therefore, add a little Na acetate before making this test, or a correspondingly large quantity of U acetate must be added To the second portion add 1 or 2 drops of NH_4OH, in the presence of cochineal, a violet coloration results This, however, is not so characteristic as the first test as many fruit colors give almost identical reactions Cochineal is not decolorized by $NaHSO_3$ either in an acid neutral or alkaline soln (differs from orchil)

As cochineal lakes often contain tin, further examination for this metal should always be made when water insoluble cochineal compounds seem to be present

24

Orchil.—This coloring matter is either sulfonated or unsulfonated Unsulfonated orchil is readily extracted by amyl alcohol from a weak acid soln, while the extraction of the sulfonated color is incomplete even from a strongly acidified soln The behavior of the color towards acids and alkalies is similar to cochineal e g The HCl produces a yellow shade and alkalies produce a bluish shade $NaHSO_3$ reduces orchil, but the color is restored by air oxidation (differing from cochineal) The characteristic property of orchil is to dye, strip, and redye wool readily

25

Caramel.—A number of tests have been developed for this coloring matter most of them being based upon the insolubility in ether, $CHCl_3$ or amyl alcohol Probably the most sensitive test is the Woodman Newhall[23] modification of Amthor's test with a slight deviation To 10–20 cc of a neutral soln of the color in a small centrifuge tube add 2 cc of 5% $ZnCl_2$ and 2 cc of 2% KOH soln stir well, and centrifugalize Pour off the liquid, and to the magma add 25 cc of boiling H_2O Mix centrifugalize, and pour off liquid Repeat this operation until the aqueous wash liquor is colorless Dissolve the precipitate with 15 cc of 10% acetic acid concentrate, neutralize carefully, and filter Divide into 2 portions To one add 3–5 volumes of paraldehyde in a 50 cc glass stoppered cylinder, and just sufficient absolute alcohol to form a homogeneous soln (avoid excess) Caramel will be indicated by the formation of a brownish precipitate on standing To the other portion of the caramel soln add an equal volume of a freshly prepared reagent consisting of phenylhydrazin hydrochloride—2 parts, Na acetate—3 parts, water—20 parts A dark brown precipitate is formed in the presence of caramel

COMMERCIAL COAL TAR FOOD COLORS[24]

PREPARATION OF SAMPLE

26

Thoroly mix and without interruption weigh out the portions required for the determinations to be made If the weighing cannot be made directly into the dish in which the determination is to be made, use weighing bottles for this purpose, placing in each a quantity approximating the weight called for and weigh immediately

27 MOISTURE

(a) *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine* —Weigh approximately 2 g of the sample in a weighed Al dish 2 inches in diameter or in a weighing bottle of the same diameter, dry in an air oven at 135° for 6 hours or overnight, cool over H_2SO_4 in a desiccator, and weigh Heat again for 1 hour, cool in the desiccator, and weigh Repeat the heating and weighing at hour intervals until the weight becomes constant Report the loss in weight as moisture

(b) *For yellow AB and yellow OB* —Proceed as directed under (a), heating the dye to 80° instead of to 135°

WATER INSOLUBLE MATTER

28 APPARATUS

Prepared Gooch crucible —Digest a good grade of retentive asbestos with HCl (1+3), wash free from acid, and elutriate to remove fine particles, pour a sufficient quantity into a Gooch crucible placed in a filter flask to make a mat ⅛ inch thick when packed Using gentle suction, pack the asbestos down evenly with a tamping rod and then remove the Gooch from the filter flask Loosen the mat around the edges with a thin narrow blade or pin, take out of Gooch, replace in an inverted position, pack down tightly, and add more of the asbestos suspension until a well packed mat 1 inch thick is obtained Wash with hot H_2O, dry, ignite, rewash, dry at 100–105°, cool in a desiccator containing $CaCl_2$, and weigh Repeat the washing, heating, and drying until constant weight is obtained

29 DETERMINATION

(a) *For amaranth, erythrosine, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, and fast green FCF* —Dissolve 5 g of dye in 200 cc of hot H_2O and allow the soln to cool to room temp Filter thru the prepared Gooch crucible, wash with cold H_2O until all dissolved dye has been removed, dry at 135°, cool in a desiccator containing $CaCl_2$, and weigh Report the increase in weight as total insoluble matter

(b) *For ponceau 3R, orange I, and indigotine* —Dissolve 5 g of dye in hot H_2O, using 250 cc for ponceau 3R and orange I, and 500 cc for indigotine, and boil, with frequent stirring, for 3 min Cool the soln to room temp and filter with moderate suction Wash with cold H_2O, dry, cool, and weigh as directed under (a)

30 NON-VOLATILE WATER INSOLUBLE MATTER

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF and indigotine —Incinerate the Gooch containing the total insoluble matter, 29, at a low red heat until all the organic matter has been volatilized Cool in a desiccator containing $CaCl_2$ and weigh

SODIUM CHLORIDE

REAGENTS

All reagents must be halogen free

Sulfur dioxide soln —Saturate ice cold distilled H_2O with sulfur dioxide Keep the soln stoppered and in a cold place

31 DETERMINATION

(a) *Applicable to ponceau 3R, amaranth, orange I, tartrazine, guinea green B, light*

green SF yellowish, fast green FCF, and indigotine —Thoroly mix 5 g of dye with 4–6 g of K_2CO_3 or Na_2CO_3 in a 50 cc Pt or Ni crucible and moisten with H_2O or 50% alcohol Cover evenly with about 1 g of the powdered carbonate dry, and ignite at low red heat until organic matter is destroyed Allow to cool and add enough H_2O to form a thin paste If any lumps are present, break them up with a glass rod in order to produce a uniform suspension Wash the mixture into a 250 cc volumetric flask with 100–150 cc of hot H_2O and allow to stand until all soluble salts are dissolved and the mixture is cold Dilute to the mark with H_2O, mix thoroly and filter thru a dry paper

Place a 200 cc portion of the filtrate in a 600 cc beaker and add enough of a 6–7% soln of $KMnO_4$ to oxidize the sulfides and produce a permanent pink color Add about 50 cc of H_2O and a slight excess of 10% $AgNO_3$ soln (usually 6–8 cc is sufficient) Partially cover the beaker with a watch glass and acidify the soln by carefully adding about 12 cc of HNO_3 Heat nearly to boiling, then add the saturated SO_2 soln Boil until any excess of SO_2 is removed, cool, filter thru a weighed Gooch crucible, wash the precipitate of AgCl with hot H_2O, dry crucible and its contents at 140–150°, cool in a desiccator, and weigh Calculate to percentage of NaCl

(b) *For erythrosine* —In a 500 cc volumetric flask dissolve 5 g of the dye in 400 cc of H_2O Precipitate the color acid by adding a mixture of 2 cc of HNO_3 and 10–20 cc of H_2O, dilute to 500 cc, mix, and filter thru a dry paper Treat 200 cc of the filtrate with slightly more 10% $AgNO_3$ soln than is required to precipitate the halogens present, add 5 cc of HNO_3, and heat to boiling Cool, collect the precipitate in a weighed Gooch crucible, wash, dry, and weigh as directed under (a) If NaI is present determine as directed under 62, and subtract the weight of AgI from the weight of the precipitate Calculate the percentage of NaCl from the net AgCl

(c) *For naphthol yellow S* —Dissolve 5 g of the dye in 250 cc of H_2O and filter if necessary Add 5 cc of HNO_3 and precipitate the chloride by adding a slight excess of 10% $AgNO_3$ soln Boil for a few min, cool, and filter thru a weighed Gooch crucible Wash, dry, and weigh the precipitate and calculate as directed under (a)

(d) *For ponceau 3R* —Dissolve 5 g of the dye in 150 cc of hot H_2O, wash into a 250 cc volumetric flask and add 25 cc of a 10% soln of $Ba(NO_3)_2$ Cool the mixture, make up to the mark, mix, and filter thru a dry paper Acidify 100 cc of the filtrate (representing 2 g of dye) with HNO_3 and treat with a slight excess of 10% $AgNO_3$ soln Collect the filtrate on a weighed Gooch crucible, wash, cool in a desiccator, weigh, and calculate as directed under (a)

(e) *For ponceau 3R, amaranth, orange I naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine* —In a Ni crucible mix 1 g of the dye with about 15 g of pure Na_2O_2 Place the cover on the crucible and start the fusion by introducing a red hot iron wire thru a hole previously bored in the cover When the reaction is complete, allow the crucible to cool (If the fusion is too rapid, repeat the entire procedure, using a larger proportion of Na_2O_2) Wash the cover into a 600 cc beaker, using 50–75 cc of H_2O Place the crucible on its side in the beaker and add H_2O until the crucible is about half covered When the mixture is dissolved, remove and wash the crucible over the beaker Neutralize the soln with HNO_3 adding 1–3 cc in excess Cool, transfer to a 6 inch porcelain evaporating dish, and add 19 cc of 0 1 *N* $AgNO_3$ soln and 5–10 cc of 10% ferric alum soln Titrate the soln with 0 1 *N* NH_4 or K thiocyanate, adding 1 or 2 drops after the red end point has been reached Add another cc of $AgNO_3$ soln for each cc of HNO_3 Return the filtrate and washing to the evaporating dish and titrate to the end point with NH_4 or K thiocyanate Calculate to percentage of NaCl

32 SODIUM SULFATE

(a) *For ponceau 3R, amaranth, orange I, tartrazine, and indigotine* —Transfer to a 250 cc volumetric flask that volume of a water soln of the sample which contains 5 g of the dye, add H_2O, if necessary, to bring the volume to 200 cc, and heat on the steam bath Add pulverized NaCl as follows For amaranth and tartrazine, 70 g, for ponceau 3R, orange I, indigotine, 50 g Stopper the flask and shake at frequent intervals for an hour (To hasten the precipitation the soln may be cooled in ice H_2O) Dilute to the mark with saturated NaCl soln, shake, and filter on a dry 18 cm paper To 100 cc of the filtrate add 200 cc of H_2O and 1 cc of HCl (1+9), heat to boiling and add a slight excess of hot 10% $BaCl_2$ soln Allow to stand overnight, filter thru a weighed Gooch crucible, wash the precipitate of $BaSO_4$ thoroly with hot H_2O, dry, ignite, cool in a desiccator containing $CaCl_2$, and weigh Calculate the weight of Na_2SO_4 equivalent to the $BaSO_4$ obtained

(b) *For erythrosine* —To such a volume of a water soln of the sample as contains 1 g of dye add H_2O, if necessary, to bring the volume to 100 cc and then add 50 cc of HNO_3 (1+49) Shake, and let settle Filter off the dye and wash once, collecting the washings with the filtrate Redissolve the dye by passing thru the filter paper as little NH_4OH (1+1) as will effect soln Neutralize the base and reprecipitate the color acid by means of the HNO_3 (1+49) Filter off the dye and wash once, collecting the filtrate and washings with those obtained after the first precipitation of color acid [A 100 cc aliquot free of the color acid 31 (b) may be substituted] Precipitate and determine the $BaSO_4$ as directed under (a)

(c) *For light green SF yellowish and fast green FCF* —Transfer to a 250 cc flask that volume of a soln which contains 5 g of the dye, add H_2O, if necessary, to bring to a volume of about 200 cc, and heat on the steam bath Add 5 g of phosphotungstic acid and shake at intervals until dissolved Then add 50 g of pure pulverized NaCl, shaking at intervals to dissolve the salt Cool, dilute to mark with saturated pure NaCl soln, shake, and filter To 100 cc of the filtrate add 200 cc of H_2O and 1 cc of HCl (1+9) and determine $BaSO_4$ as directed under (a)

(d) *For naphthol yellow S* —To a volume of a water soln of the dye that contains 5 g of the sample in a 500 cc volumetric flask, add H_2O, if necessary, to bring to a volume of about 300 cc Add hot saturated KCl soln to bring to the 500 cc mark Shake frequently until practically all the dye is precipitated After the mixture is cold dilute with H_2O to 500 cc Shake, and filter thru a dry paper Complete the determination as directed under (a) beginning with "To 100 cc of the filtrate add 200 cc of H_2O and 1 cc of HCl (1+9) "

(e) *For guinea green B* —Transfer to a 500 cc volumetric flask that volume of a water soln of the sample which contains 5 g of the dye, and add H_2O, if necessary, to bring the volume to 400 cc Add 8 cc of NH_4OH and 125 g of NaCl (free from sulfates), and dilute to 500 cc with a saturated soln of NaCl Shake vigorously to precipitate the dye and filter thru a dry paper Neutralize 200 cc of the filtrate with HCl (1+9) and add 5 cc in excess Complete the determination as directed under (a) beginning with "heat to boiling, and add a slight excess of hot 10% $BaCl_2$ soln "

33 SULFATED ASH

(a) *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine* —Weigh accurately in a weighing bottle about 5 g of the dye and transfer it to a Pyrex Kjeldahl flask, washing out the weighing bottle with a little H_2O Destroy the organic matter to a convenient extent by digestion, using 15 cc of H_2SO_4 and adding HNO_3 as required As the bulk of the HNO_3 is driven off, lower the flame to avoid reaction on

the glass Transfer the mixture to a weighed Pt dish and heat over a ring burner, using at first a low flame at a safe distance below the dish, increasing the flame, and bringing it closer to the dish by gradual steps Thus continue the destruction of the organic matter and the volatilization of the acids Continue the heating until the production of acid fumes decreases If C remains, remove the flame, let the mass cool a little, and add H_2SO_4, dropwise, until the mass is moistened Repeat the treatment until the C is burned off and the ash is white or reddish Heat carefully with a blast lamp until fusion takes place with the production of a clear liquid free from bubbles Cool in a desiccator and weigh After deducting the weight of Na_2SO_4 equivalent to the inorganic Na salts (chlorides, sulfates, carbonates, etc) found in the other determinations, calculate to percentage of metallic Na combined in the dye

(b) *For yellow AB and yellow OB* —In a weighed Pt dish weigh 5 g of the dye, heat at a low temp until most of the dye has been volatilized, moisten the residue with H_2SO_4, and complete the determination as directed under (a) beginning with ' Repeat the treatment until the C is burned off "

HEAVY METALS

34

(a) *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine* —Moisten the sulfated ash obtained under 33 with a few cc of HCl and evaporate to dryness on the steam bath Warm the residue with 20 cc of HCl (1+19) until all soluble material has dissolved, transfer to a 100 cc volumetric flask, dilute to 100 cc mix, and filter thru a dry paper Reserve two 40 cc aliquots for the determination of Al, Ca, Fe and Mg Pour 20 cc of the filtrate into a test tube and pass in a washed stream of H_2S for 30 min No turbidity other than that due to precipitated S should appear If a colored precipitate is formed, filter and test it for Pb Cu and Sn Make the filtrate slightly alkaline with NH_4OH (1+1) A white precipitate indicates Zn a marked coloration indicates that the quantity of Fe should be determined

(b) *For yellow AB and yellow OB* —The color of the sulfated ash obtained under 33 shows whether it is mainly Fe_2O_3 or SiO_2 In either case fuse it with 1 g of K_2CO_3 until the silicates have been decomposed Moisten the residue with 2 or 3 cc of HCl, evaporate to dryness on the steam bath, and treat as directed under (a) If only a trace of Fe_2O_3 or SiO_2 is present in the original ash the fusion with K_2CO_3 may be omitted

LEAD[a]

(Applicable to all permitted dyes)

REAGENTS

35

(a) *Ammonium acetate* —Dissolve 200 g of pure NH_4-acetate in H_2O and dilute to 500 cc

(b) *Ammonium acetate soln* —Dilute 50 cc of the strong NH_4-acetate to 500 cc

DETERMINATION

36

Place 5 g of the dye in a tall form, 500 cc Pyrex beaker cover with a watch glass add 15 cc of HNO_3 and let boil or heat gently till the rapid evolution of brown fumes has ceased Add 15 cc of H_2SO_4 and continue the heating Add small quantities (1–2 cc) of HNO_3 at intervals until the organic matter is destroyed and the soln is colorless or at most a pale yellow Continue the heating with the evolution of dense

white fumes, until a very small quantity (3–5 cc) of soln remains in the beaker Cool the soln, which should form white or pale yellow crystals, and add 15–20 cc of H_2O Re evaporate the soln thus formed down to white fumes, cool, take up in 100 cc of H_2O, add 100 cc of 95% ethyl alcohol, and let stand overnight Filter out the precipitate of $PbSO_4$, which may be present in such a small quantity as to escape detection with the naked eye, and wash thoroly with 50% ethyl alcohol (about 100 cc is the usual amount of 50% alcohol used) (Two 9 cm C S & S No 590 filter papers, or a suitable, fritted glass crucible, have been found satisfactory for retaining the $PbSO_4$)

Place the filter paper in a small beaker, add 20 cc of strong NH_4 acetate, and heat to boiling, breaking up the paper with a glass rod Filter thru a C S & S No 590 9 cm paper, or thru a fritted glass crucible, into a 100 cc colorimeter tube and wash with dilute NH_4 acetate soln until the 50 cc mark is reached If desired, filter into a 50 cc volumetric flask and take an aliquot portion to be used in the colorimeter tube Prepare standards containing known quantities of Pb for comparison To these add the same quantity of NH_4 acetate as was used with the sample and dilute all tubes to a definite volume with H_2O To each tube add 2 or 3 drops of glacial acetic acid and 10 cc of freshly prepared H_2S water Shake the tubes to insure thoro mixing and estimate the quantity of Pb by comparison with the standards Blanks should not be over 4 p p m when using the best reagents obtainable

37 IRON, ALUMINUM CALCIUM AND MAGNESIUM[26]

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, yellow AB yellow OB, guinea green B, light green SF yellowish, fast green FCF and indigotine—To one of the two portions reserved under 34, add 5 g of NH_4Cl and neutralize with NH_4OH, boiling to drive off any excess If the precipitate is very slight, it may be disregarded, otherwise, filter thru a quantitative paper, wash (reserving the filtrates and washings), and ignite paper and precipitate in a weighed crucible Weigh the mixture of Fe_2O_3 and Al_2O_3 Place the mixed oxides in a 500 cc Erlenmeyer flask and dissolve in a HNO_3 and HCl mixture, boiling to drive off Cl Add H_2O to bring the volume to about 75 cc and add NH_4OH to incipient precipitation Dissolve the precipitate with as little HCl as possible, cool, and titrate the ferric iron present with 0 1 *N* $TiCl_3$ soln, 46, using 5 g of NH_4CNS as indicator Calculate the Fe as Fe_2O_3 To calculate the quantity of Al_2O_3, deduct the weight of ferric oxide from the total weight of mixed oxides From the weights of the oxides calculate the percentage of metallic iron and Al Pass a washed stream of H_2S into the alkaline filtrate from the Fe and Al hydroxides A white precipitate indicates the presence of Zn

To the other reserved portion add 250 cc of H_2O to insure a low concentration of Mg if present Heat to boiling and add 3 5 g of NH_4Cl and enough NH_4OH soln (1+99) to make the soln barely alkaline Filter off the precipitated hydroxides of Fe and Al Wash and discard the precipitate Heat the combined filtrate and washings to boiling and add 1 g of NH_4 oxalate After cooling and letting stand for an hour, filter thru an asbestos mat prepared on a small Witt plate in a glass funnel and wash with very little H_2O reserving the combined filtrate and washings Place the mat in a beaker, add 100 cc of H_2O and 2 cc of H_2SO_4, heat gently until the Ca-oxalate dissolves and titrate with 0 1 *N* $KMnO_4$ soln Calculate as metallic Ca

Heat to boiling the reserved filtrate and washings and add a *N* soln of $NaNH_4HPO_4$ until there is no further precipitation While stirring add about ⅓ the volume of NH_4OH (1+9) Let stand 3 hours, filter thru an ashless paper, and wash with NH_4OH (1+49) Ignite the filter and precipitate in a weighed crucible, cool in a desiccator, and weigh the $Mg_2P_2O_7$ Calculate as metallic Mg

ARSENIC

I By Direct Precipitation

REAGENTS

38

(a) *Ammonium hydroxide*.—Arsenic free and containing not less than 26% by weight of NH_3

(b) *Hydrochloric acid* (1+3).—Should be As free

(c) *Sodium phosphate soln*.—As free and containing 100 g of the crystallized salt per liter or an equivalent quantity of phosphoric acid

(d) *Magnesia mixture*.—As free and containing 55 g of hydrated $MgCl_2$, 35 g of NH_4Cl, and 88 cc of NH_4OH per liter

The other reagents and solns used are described under XXIX, 1

APPARATUS

39

The apparatus used is described under XXIX, 2

DETERMINATION

40

(a) *For amaranth, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, and fast green FCF*.—Dissolve 10 g of the dye in 250 cc of H_2O and add 10 cc of strong Br water. Make the mixture alkaline with 1–2 cc of the strong NH_4OH, then add 20 cc of the Na phosphate soln and a slight excess of the Mg mixture. (The quantity of Mg mixture used must be from 1–5 cc in excess of that required to precipitate the phosphate completely, as ascertained previously by experiment.) Add the Mg mixture to the dye mixture slowly, stirring the soln during the addition. Add 10 cc of the NH_4OH and allow the mixture to stand for at least 30 min. Filter thru an 18 cm paper and wash with NH_4OH (1+9) until practically all dye is removed, then wash with about 5 cc of H_2O. Allow the filter containing the washed precipitate to drain for 15–30 min. to remove most of the adhering liquid. Finally dissolve the Mg NH_4 phosphate and arsenate by pouring 40 cc of the HCl (1+4) over the filter in small portions and letting it drain into the generator bottle. Complete the determination as directed under XXIX, 4, beginning with "add 5 cc of the KI reagent"

(b) *For erythrosine*.—Dissolve 18 g of the dye in 425 cc of H_2O and add 5 cc of Br water and 20 cc of the HCl (1+3). Mix, filter, and treat 250 cc of the filtrate (corresponding to 10 g of dye) with the strong NH_4OH soln using a quantity sufficient to render it slightly alkaline (usually about 5 cc). Complete the determination as directed under (a), beginning with "then add 20 cc of the Na phosphate soln"

II After Treatment with Nitric Acid

41

(a) *For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, and indigotine*.—Place 12.5 g of the powdered dye in a 600 cc Pyrex beaker, add 2 cc of the strong HNO_3, and, if the dye tends to form a clot or cake, stir the mixture thoroly. Boil for about 5 min., add 150–200 cc of H_2O, and dilute the mixture to 250 cc in a volumetric flask. Pour the mixture back into the beaker, allow to stand for a few min., and then filter thru an 18 cm paper. Treat 200 cc of the filtrate (corresponding to 10 g of dye) with the strong NH_4OH using a measured quantity sufficient to make the soln slightly alkaline (approximately 25 cc). Complete the determination as directed under 40 (a), beginning with "then add 20 cc of the Na phosphate soln"

(b) *For yellow 4B and yellow OB*.—In a 400 cc beaker mix thoroly 12.5 g of the powdered dye with 200 cc of HNO_3 (1+9), heat to boiling for 5–10 min., allow to

cool, add 25 cc of the strong NH_4OH, dilute to 250 cc in a volumetric flask, and filter Determine the As in a 200 cc portion of the filtrate (corresponding to 10 g of dye) as directed under 40 (a), beginning with "then add 20 cc of the Na phosphate soln "

42 *III As Total Arsenic*

For ponceau 3R, amaranth, erythrosine, orange I, naphthol yellow S, tartrazine, yellow AB, yellow OB, guinea green B, light green SF yellowish, fast green FCF, and indigotine —Weigh 10 g of the dye into a 600 cc Kjeldahl flask or tall 600 cc beaker provided with a cover Treat with 15 cc of the concentrated H_2SO_4 and 25 cc of the HNO_3 and digest slowly under a hood until the HNO_3 has been decomposed or volatilized and the mixture turns dark Cautiously add a few cc of HNO_3 to the hot mixture, which will again become light yellow or orange, and heat to charring Repeat the HNO_3 treatment until the soln no longer shows a tendency to darken and remains yellow or colorless when evaporated until SO_3 fumes are evolved Allow the completely digested mixture to cool, add 200 cc of H_2O, and make slightly alkaline with NH_4OH Determine the As in the soln as directed under 40 (a), beginning with "then add 20 cc of the Na phosphate soln " The comparatively large quantities of reagents necessary for the destruction of the organic matter will usually contain appreciable quantities of As, for which correction must be made by determinations on blanks

ETHER EXTRACTIVES

43 REAGENTS

Washed ether —Wash 1 liter of ether with 3 successive 150 cc portions of H_2O immediately before using

44 DETERMINATION

(a) *For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine* —Place in a separatory funnel that volume of a soln that contains 10 g of the dye and add water, if necessary, to bring the volume to 200 cc, and, in the case of indigotine, to 500 cc Extract with 2 successive 100 cc portions of the washed ether, shaking for 1 min during each extraction Remove the ether by decantation into a clean funnel and rinse the first funnel with 5 cc of ether, decanting into the second funnel Reserve the color soln Wash the combined extracts with 20 cc portions of H_2O until the washings are colorless Decant the ether into a beaker, rinse the funnel with 5 cc of ether, and decant into the same beaker Place the beaker in a dust free atmosphere, allow the ether to evaporate to a volume of 50 cc, and transfer to a weighed flat bottomed 100 cc dish, previously dried to constant weight over H_2SO_4 in a desiccator Rinse the beaker with 5 cc of ether and drain into the same dish Let the remainder of the ether evaporate and dry over H_2SO_4 to constant weight The result represents the neutral extract

To the reserved color soln, add 2 cc of a 10% NaOH soln and extract and rinse with ether Reserve the color soln Wash the combined ether extracts and rinsings with 20 cc portions of the dilute NaOH soln until the washings are colorless Evaporate the ether, dry, and weigh The result represents the alkaline extract

To the color soln reserved from the alkaline extraction, add twice the volume of HCl (1+3) necessary to neutralize Repeat the previous procedure, but do not reserve the color soln Wash the ether extract with the dilute HCl until the washings are colorless The result represents the acid extract

(b) *For erythrosine*—Determine as directed under (a), omitting the acid extrac

tion

45

SULFUR

For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, and indigotine—(1) Determine the S content in 0 2–0 3 g of the dye by the Carius method [27] using 3 cc of fuming HNO_3 and heating the sealed tube to 300° for at least 8 hours, or (2) Place about 0 2 g of the sample in a Parr calorimetric bomb and mix thoroly with approximately 10 g of Na_2O_2 Add a few mg of S free sugar if necessary to aid in ig niting the mass Close the bomb and ignite When cool open the bomb, place it in a 400 cc beaker, and cover the beaker with a watch glass Dissolve the residue by adding warm H_2O thru the lip of the beaker until the bomb is covered Acidify the soln cautiously with dilute HCl and filter, if ne cessary Determine the H_2SO_4 as directed under 32 (a), beginning with 'heat to boiling,' and add a slight excess of hot 10% $BaCl_2$ soln Deduct the S equivalent to the Na_2SO_4 determined un der 32

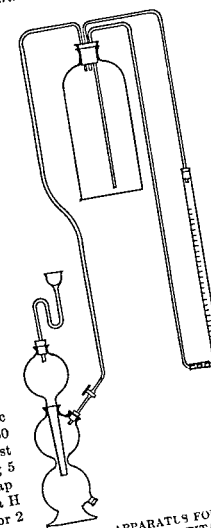

FIG 20—APPARATUS FOR TI TRATION WITH TITA NIUM TRICHLORIDE

COLOR ACID AND DYE

I By Titration with Titanium Trichloride

46

REAGENT

Standard titanium trichloride soln—To 200 cc of the commercial 15% soln of $TiCl_3$, add 150 cc of HCl and dilute to 2 liters Titrate against 10 cc of 0 1 *N* ferric ammonium sulfate, using 5 g of NH_4CNS as an indicator Make the soln ap proximately 0 1 *N*, place in a container with a H atmosphere provision [28] and allow to stand for 2 days for absorption of residual O Standardize against 20 cc of 0 1 *N* ferric ammonium sulfate using 5 g of NH_4CNS as indicator and titrating in a 500 cc Erlenmeyer flask in a protecting stream of CO_2 Preserve in an atmosphere of H [28

STANDARDIZATION OF SOLUTION

Method I—Prepare a liter of 0 1 *N* $Fe_2(SO_4)_3$ by dissolving ingot iron, Bureau of Standards Sample 55, in dilute H_2SO_4, using 30 cc of the concentrated acid Dilute to about 400 cc, adding slowly with stirring a soln of pure $KMnO_4$ (3 16 g dissolved in about 200 cc H_2O) until a faint but perceptible reddish tint results The last few cc should be added dropwise Cool and dilute to 1 liter Measure 20 cc of the 0 1 *N* $Fe_2(SO_4)_3$ into a 500 cc flask, pass in a strong stream of CO_2 and add the $TiCl_3$ rapidly until near the end point Add 5 g or pure NH_4CNS and resume the addition of $TiCl_3$ carefully until the red color just disappears

Method II—Make up a 0 1 *N* soln of $KMnO_4$ and standardize carefully, using Na_2 oxalate Bureau of Standards Sample 40 according to the directions supplied with

the sample Weigh 3 g of ferrous ammonium sulfate and transfer to a 500 cc flask Introduce a stream of CO_2 and add 50 cc of recently boiled H_2O and 25 cc of 40% (by weight) H_2SO_4 Then, without interrupting the current of CO_2, add rapidly 40 cc of the standardized $KMnO_4$ Add $TiCl_3$ until near the calculated end point Then add quickly 5 g of NH_4CNS, and complete the titration Run a blank on 3 g of ferrous ammonium sulfate, using the same quantities of H_2O, acid, NH_4CNS, and the current of CO_2

INDICATOR

For many dyes the $TiCl_3$ titration end point is indicated by a sharp decolorization For some dyes the change is so gradual that an excess of $TiCl_3$ (not more than 0 3 cc of approximately 0 1 *N* soln) is required, and a suitable standard soln of some other dye must be used for the back titration, methylene blue serving well for this purpose In other cases it is better to use an indicator which is reduced after the original dye has reacted with the $TiCl_3$ Thus a known quantity of light green SF yellowish serves well for this purpose

47

Prepare a dye solution of such strength that 50 cc will require approximately 20 cc of the standard $TiCl_3$ soln for its reduction See table below

Quantities of color acids and of pure coal tar dye equivalent to 1 cc of 0 1 N titanium trichloride solution

DYE	MOLECULAR WEIGHT OF COLOR ACID	COLOR ACID EQUIVALENT TO 1 CC 0 1 *N* $TiCl_3$	DYE EQUIVALENT TO 1 CC 0 1 *N* $TiCl_3$
Ponceau 3R	450 4	0 01126	0 01236
Amaranth	538 4	01346	01511
Orange I	328 3	008207	008756
Naphthol yellow S	314 2	002618	002985
Tartrazine	468 3	01171	01336
Guinea green B	668 5	03342	03453
Light green SF yellowish	748 7	03743	03963
Fast green FCF	764 5	03822	040423
Indigotine	422 3	02112	02332

48

DETERMINATION

(a) *For amaranth and ponceau 3R* —Place in a 500 cc Erlenmeyer flask a volume of soln that corresponds to 20 cc of 0 1 *N* $TiCl_3$ Add 10 g of Na-citrate and H_2O if necessary to bring the volume to 100 cc Introduce a stream of CO_2 and titrate with the standardized $TiCl_3$, keeping the CO_2 flow continuous to the end

(b) *For orange I, tartrazine and indigotine* —Proceed as directed under (a), substituting 15 g of Na acid tartrate for Na citrate and bringing the volume to 100 cc First completely dissolve the tartrate by boiling, then add the dye soln, and boil again under CO_2 Run blanks on 15 g of the acid tartrate using the above volume of H O and a soln containing 1 mg of the dye concerned

(c) *For guinea green B, light green SF yellowish, and fast green FCF* —In a 1 liter Erlenmeyer flask, dissolve 30 g of Na-acid tartrate in 200 cc of H O, boil to expel air, and under CO_2, cool to 85° Add 50 cc of the dye soln prepared as directed under 47 and 50 cc of H_2O, and titrate with the standard $TiCl_3$ soln at 60–70°

(d) *For naphthol yellow S* —Proceed as directed under (a), using as indicator that volume of light green SF yellowish standardized soln (freshly made) that contains 10 mg of dye Run a blank on the tartrate, light green SF yellowish, and H_2O

II By Precipitation[23]

49 *For erythrosine* —To that volume of a water soln of the sample that contains 0 25 g of dye, add, if necessary, sufficient H_2O to bring the volume to 100 cc Then add 5 cc of HNO_3 of approximately 0 6 *N* strength and filter thru a weighed Gooch cru cible Wash thoroly with 0 5% HNO_3 and finally with not more than 10 cc of H_2O The precipitate should not be allowed to cake in the crucible until the washing has been completed Dry to constant weight at 135

PURE COAL TAR DYE

I By Direct Titration with Standard Titanium Trichloride Solution

50 *For yellow AB and yellow OB* —Dissolve 15 g of Na acid tartrate in 100 cc of H_2O and add 0 1 g of dye dissolved in 100 cc of 95% alcohol Titrate with the standard $TiCl_3$ soln, 46, under CO_2 using as an indicator 10 mg of light green SF yellowish from a fresh standardized soln as directed under 48 (d) Run a blank as directed under 48 (d), including also the 100 cc of 95% alcohol 1 cc of 0 1 *N* $TiCl_3$ = 0 006180 g of yellow AB and 0 006030 g of yellow OB Calculate the percentage of pure dye

II By Precipitation

51 *For erythrosine* —Multiply the percentage of color acid as obtained under 49 by the factor 1 074

III By Titration With Potassium Permanganate

52 *For indigotine* —Take that volume of a freshly made water soln of the dye which contains 0 01 g of dye and dilute if necessary to 400 cc Add 2 cc of H_2SO_4 and titrate against standard approximately 0 02 *N* $KMnO_4$ soln The end point is shown by the production of a clear yellow color The titer of the standard soln must be fixed by titration against a freshly made soln of indigotine of known purity the same conditions of concentration and acidity being maintained

MATTER INSOLUBLE IN CARBON TETRACHLORIDE

53 *For yellow AB and yellow OB* —In a 100 cc beaker mix 5 g of the dye with 50 cc of CCl_4 stir and heat to boiling Wash a Gooch crucible prepared as directed under 28 with CCl_4 and heat at 105° to constant weight Filter the hot dye soln thru the crucible transferring to it the residue in the beaker and wash with five 10 cc portions of CCl_4 Dry at 100° 110° and weigh

WATER EXTRACTIVES

54 *For yellow AB and yellow OB* —(a) *Neutral extractive* —Place 5 g of the well powdered dye in a 500 cc Erlenmeyer flask or wide mouthed bottle add 200 cc of H_2O stopper and mix by shaking vigorously several times during 2 hours Filter the mixture re crying the dye and evaporate 100 cc of the filtrate on a steam bath in a weighed Pt dish Dry in an oven at 100–105° cool and weigh The result repre sents the neutral extractive Test small portions of the remainder of the filtrate for chlorides sulfates and nitrates If more than traces are present make the proper analyses on aliquot portions of the filtrate

(b) *Acid extractive* —Dissolve the residual dye reserved under (a) in 100 cc of benzene and in a separatory funnel extract with 2 successive 2 cc portions of HCl (1 + 9) Combine the extracts and wash once with 2 cc of benzene Draw off the acid extract into a weighed Erlenmeyer flask Discard the benzene and wash the funnel with 10 cc of HCl (1 + 9) adding this wash ing to the extract in the flask Evaporate to

dryness on the steam bath, dry at 100–105°, cool in a desiccator containing $CaCl_2$, and weigh The result represents the acid extractive

MELTING POINT

For yellow 4B and yellow OB —

55 APPARATUS

The apparatus, Fig 21, consists of a tube about 15 cm long and 3 5 cm in internal diameter, with a bulb of 5 cm internal diameter Fill with glycerin to about the height indicated Fit the tube with a cork stopper carrying a glass tube (*A*), 5 mm in diameter, which reaches nearly to the bottom of the bath, an ordinary test tube (*B*) in which a thermometer (*C*) is suspended by means of a rubber stopper in such a manner that the Hg column is wholly within the tube and the Hg bulb equidistant from its walls, a long stemmed thermometer (*D*), supported so as to reach a short distance below the tube (*B*), and an outlet tube (*E*) to permit the escape of air and vapor

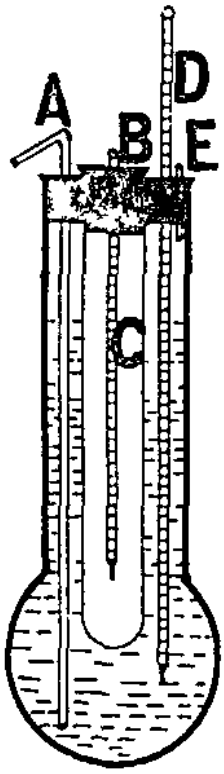

FIG 21 —MELTING POINT APPARATUS

56 DETERMINATION

To a capillary tube of 1 mm or smaller internal diameter sealed at one end, transfer a small portion of the sample by inserting into the sample the open end of the capillary, removing, inverting, and gently tapping until the well packed substance fills the bottom of the tube to a height of 2–4 mm Attach the capillary tube to the thermometer *C* by means of a small rubber band, so that the sample is placed at about the middle of the Hg bulb Replace the thermometer in the tube, connect the tube *A* to the air blast, and force a fairly rapid stream of air bubbles thru the bath Raise the temp of the bath rapidly to within 5° of the approximate melting point of the sample Keep the temp constant until the thermometer reading is within 1° of that of the bath Then raise the temp slowly until the melting point is observed On approaching within 0 5° of the melting point, the substance darkens, the true melting point is indicated by the formation of a meniscus on the upper surface When this condition is observed hold the temp as nearly constant as possible until the whole of the sample has liquefied

LOWER SULFONATED DYES

57 REAGENTS

(a) *Titanium trichloride soln* —0 1 *N* Prepare and preserve as directed under 46

(b) *Salt acetate soln* —Dissolve 125 g of NaCl in H_2O, add 12 cc of glacial acetic acid and a soln of 13 6 g of Na acetate, and dilute to 500 cc

58 PREPARATION OF SOLUTION

(a) *For amaranth, tartrazine ponceau 3R, and indigotine* —Prepare a water soln of such strength that 50 cc will contain 0 2 g of the dye

(b) *For light green SF yellowish, and fast green FCF* —Prepare a water soln of such strength that 10 cc will contain 0 1 g of the dye

DETERMINATION

59 (a) *For amaranth and tartrazine* —To 50 cc of the soln prepared as directed under 58 (a), add 1 cc of HCl Extract the lower sulfonated dye by shaking the soln successively in 3 separatory funnels, each containing 50 cc of amyl alcohol Wash the amyl alcohol extracts by shaking successively with three 50 cc portions of 0 25 *N* HCl, passing each acid portion thru the 3 funnels in the order used for the original soln (1 volume of HCl, diluted with 50 volumes of H_2O yields a soln of about 0 25 *N* strength) Dilute the amyl alcohol in each funnel with 100 cc portions of gasoline (sp gr 0 65), and remove the lower sulfonated dye by washing with several 10 cc volumes of H_2O, passing each portion thru the 3 funnels in an order the reverse of that previously followed

Determine the dye in the water extract by titration against the standard $TiCl_3$ soln, using 15 g of Na acid tartrate and having a volume of 100 cc Run a blank de termination on all reagents, using 1 mg of the dye concerned Calculate the result to percentage of fast red E in amaranth and to fast yellow G in tartrazine 1 cc of 0 1 *N* $TiCl_3$ soln = 0 01256 g of fast red E and 0 01081 g of fast yellow G If the quan tity of dye is very low, it may be determined colorimetrically, amaranth or tartra zine, as appropriate, being used as standard

(b) *For ponceau 3R* —Proceed as directed under (a) substituting a mixture of equal volumes of amyl alcohol and gasoline (sp gr 0 65) in place of the amyl alcohol and running the blank with 1 mg of ponceau 3R Calculate the result to percentage of Na trimethyl benzene azo B naphthol sulfonate, using the factor 0 009807

(c) *For indigotine* —Proceed as directed under (a), substituting 0 4 cc of acid for 1 cc, washing with 0 0625 *N* instead of 0 25 *N* acid, and running the blank with 1 mg of indigotine Calculate the result to percentage of Na indigo monosulfonate using the factor 0 01821

(d) *For light green SF yellowish and fast green FCF* —To 10 cc of the dye soln pre pared as directed under 58 (b), add 40 cc of the salt acetate soln and extract succes sively in 3 separatory funnels, each containing 50 cc of amyl alcohol Wash the ex tracts with three 50 cc portions of the salt acetate soln, passing each wash portion successively thru the 3 funnels in the order used for the original extractions Remove the dye from the alcohol as directed under (a) and determine colorimetrically by comparison with a standard guinea green B soln of approximately the same strength for light green SF yellowish, and by comparison with a standard soln of fast green FCF, for the percentage of subsidiary dye Report as percentage of guinea green B

BOILING RANGE OF CUMIDINE FROM PONCEAU 3R

60 (a) Dissolve 60 g of the dye in a 600–700 cc beaker with about 450 cc of boiling H_2O, and add the hot soln very slowly to a warm (60–80°) soln of 100 g of $SnCl_2$ in 100 cc of HCl in a tall 1200 cc beaker Add the dye soln in 10–20 cc portions waiting after each addition until the mixture is a pale brown otherwise the dye will be precipitated, in which case it can be reduced only with difficulty As reduction proceeds and the soln becomes more dilute heat to boiling taking care that the mixture does not boil over after each addition of dye, as some heat is generated by the reaction After all the dye has been added and reduced, allow the mixture to cool, and make alkaline by the addition of about 75 g of NaOH dissolved in 150–200 cc of H_2O

Cool the alkaline mixture and extract the cumidine by shaking it with three 200 cc portions of ether Combine the ether extracts thus obtained and wash with H_2O until the alkali and salts are removed Evaporate the solvent on the steam bath, but

avoid such prolonged heating as may tend to volatilize the base Transfer the residue of crude cumidine to a small side neck flask and distil it, carefully avoiding over heating Observe the range within which the substance volatilizes

(b) Proceed as directed under (a) to the directions for the extraction with ether Then steam distil the alkaline mixture until no more oil is carried over Separate the oil layer and extract the water layer with two 150 cc portions of ether Add the extracts to the oil layer and wash the mixture with successive 10 cc portions of H_2O until the alkali and salts are removed Evaporate the solvent and complete the determination as directed under (a)

61 ISOMERIC AND SIMILAR DYES IN AMARANTH

Take a volume of a water soln of the sample that contains 0 1 g of the dye and dilute, if necessary, to 40 cc with H_2O Add 10 cc of 0 1 *N* benzidine soln (9 2 g of base per liter in 0 5 *N* HCl), mix well, and allow to stand exactly 2 min Filter thru a fluted paper and dilute 10 cc of the filtrate to 100 cc Compare this soln colorimetrically with a standard amaranth soln containing 0 4 mg of the dye per 100 cc The soln of the amaranth to be tested may be used in making the standard soln If, after the benzidine treatment, the soln obtained is not more intensely colored than the standard soln, the proportion of isomeric dyes may be considered to be below 1 5%

62 SODIUM IODIDE

For erythrosine —Dilute to approximately 400 cc that volume of a water soln of the sample that contains 5 g of the dye and add a mixture of 2 cc of HNO_3 and 10-20 cc of H O Dilute to exactly 500 cc, mix, and filter thru a dry paper Place 200 cc of the filtrate in a porcelain casserole and make slightly alkaline with 10% NaOH soln Add approximately 20 cc of 7% $KMnO_4$ soln, mix and add 10 cc of HNO_3 Place on a steam bath and evaporate to dryness Add 5 cc of 7% $KMnO_4$ and 5 cc of HNO_3 and again evaporate to dryness Then add approximately 50 cc of H_2O, 5 cc of HNO_3 and 25–30 cc of a saturated soln of SO_2 Stir frequently, breaking up any lumps, until the hydrated oxide of Mn has dissolved Filter, wash the paper with H_2O add to the combined filtrate and washings an excess of 10% $AgNO_3$ soln and boil until the SO has been expelled Collect the precipitate on a weighed Gooch crucible wash with hot H_2O, dry and weigh Calculate as percentage of NaI

63 IODINE ORGANICALLY COMBINED

For erythrosine —Place in a porcelain casserole that volume of a water soln of the sample that contains 0 3–0 4 g of the dye Add 5 cc of a 10% NaOH soln and 35 cc of a 7% soln of pure $KMnO_4$ and mix Partially cover the vessel with a watch glass and add 10 cc of HNO_3 Place on the steam bath and keep covered until spattering ceases remove the watch glass and allow evaporation to proceed to dryness (Care should be taken to prevent access of reducing gases or vapors to the mixture) Treat the residue with 5 cc of 7% $KMnO_4$ and 5 cc of HNO_3 and again evaporate to dryness Add approximately 50 cc of H_2O, 5 cc of HNO_3, and 40 cc of a saturated soln of SO_2 and let stand with occasional stirring (breaking up the lumps with a glass rod) until the hydrated oxide of Mn has dissolved

Filter, wash the paper thoroly with H_2O, add an excess of 10% $AgNO_3$ to the combined filtrate and washings, and boil until SO_2 has been expelled and the AgI has flocculated Collect the precipitate on a weighed Gooch crucible, wash with hot H_2O dry, and weigh Calculate as percentage of free I and from the result subtract the percentage of the I found as NaI 62 This result is the I organically combined

TOTAL HALOGENS

64 *For erythrosine* —Mix 0 5–1 g of the dye with 4 g of K_2CO_3 and moisten to a paste with 50% alcohol Dry, cover with a layer of dry K_2CO_3 and ignite at a low red heat thoroly Allow to cool, moisten with a few drops of H_2O, and break up the charred mass filter Wash the insoluble matter until the washings no longer react with $AgNO_3$, then acidify the filtrate and washings with HNO_3, using an excess equivalent to 5 cc of the strong acid and precipitate the halogens with 10% $AgNO_3$ soln Collect the precipitate on a weighed Gooch crucible, wash, dry and weigh Compare with the sum of the results obtained in the separate halogen determinations

SODIUM CARBONATE

65 *For erythrosine* —Determine total CO_3 as directed under XV, 4, using a 10 g sample Calculate and report as Na_2CO_3

ORANGE II IN ORANGE I

66 To a volume of a water soln of the sample that contains 1 g of dye add H_2O if necessary to bring the volume to 100 cc and 10 cc of HCl Extract this soln by shaking successively in three 500 cc separatory funnels each containing 100 cc of amyl alcohol Wash each of the 3 amyl alcohol extracts by means of six 100 cc portions of 1% Na_2CO_3 soln (53 g of anhydrous Na_2CO_3 to the liter) passed successively thru the funnels in the order first used (In washing the acidified amyl alcohol solns, shake gently at first keeping the funnel upright and unstoppered until the evolution of CO_2 is slow enough to permit more vigorous shaking) In the same manner wash the extracts in the second and third funnels with 2 more 100 cc portions of the Na_2CO_3 soln and wash the extract in the third funnel with 2 additional portions of the carbonate soln Dilute the amyl alcohol solns by extracting completely with the requisite number of 10 cc portions of H_2O passed thru the funnels reversing the order previously used Bring the volume to 100 or 150 cc by adding H_2O add about 10 g of Na acid tartrate and titrate with standard $TiCl_3$ soln 1 cc of 0 1 N $TiCl_3$ = 0 0087 g of orange II

MARTIUS YELLOW IN NAPHTHOL YELLOW S

67 Dissolve 5 g of the dye in 150 cc of H_2O add 5 cc of HCl and shake vigorously in a separatory funnel for 1 min with 50 cc of gasoline (sp gr 0 69) Separate the solns and extract the aqueous liquid again with 25–30 cc of the solvent Combine the portions of gasoline decant into a clean separatory funnel and wash with four 25 cc portions of 0 2% NaOH soln Neutralize the martius yellow by shaking with a few portions of 5% NaOH soln and titrate against standard $TiCl_3$ soln as directed under 48 (d) 1 cc 0 1 N $TiCl_3$ = 0 002131 g of martius yellow

Very small quantities (less than 0 1%) may also be determined colorimetrically in neutral or slightly alkaline soln) by comparison with a standard naphthol yellow S soln the tinctorial power of which is considered to be 9/10 that of martius yellow

TOTAL NITROGEN

68 (a) *For ponceau 3R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green SF yellowish, fast green FCF, indigotine, yellow AB and yellow OB* Proceed as directed by Dumas[1]

XXII DAIRY PRODUCTS

MILK

1 COLLECTION OF SAMPLE—OFFICIAL

The quantity of sample required depends upon the number of determinations to be made For the usual analysis collect 250–500 cc (½ pint) of sample, for the fat determination only, 50–60 cc (approximately 2 fl oz) will suffice

In the case of bottled milk collect one or more bottles as prepared for sale In sampling bulk milk thoroly mix by pouring from one clean vessel into another 3 or 4 times If this procedure is impracticable, thoroly stir the milk for at least 30 seconds with a suitable appliance long enough to reach to the bottom of the container If cream has formed on the milk, continue the mixing until all cream is detached from the sides of the vessel and evenly emulsified thruout the liquid

Place the samples in non-absorbent, air tight containers and keep them in the cold, but at a temp above freezing, until ready for examination When transported by mail, express, or otherwise, completely fill the containers, tightly stopper, and mark for identification A suitable quantity of preservative (HgCl , $K_2Cr_2O_7$, or HCHO) may be used unless the presence of the preservative is objectionable in connection with physical or chemical tests to be applied in addition to the determination of fat

2 PREPARATION OF SAMPLE—OFFICIAL

Before withdrawing portions for analytical determinations, bring the sample to a temp of 15–20 and mix thoroly by pouring into a clean receptacle and back until a homogeneous mixture is assured If lumps of cream do not completely disappear, warm the sample to about 38°, mix thoroly, then cool to 15–20° In case a measured volume is required in a determination, bring the temp of the sample to 20° before pipetting

3 SPECIFIC GRAVITY—TENTATIVE

Determine specific gravity at 15 6/15 6° by means of a pycnometer (cf XVII, 24, or by means of a standardized hydrometer

4 ACIDITY—TENTATIVE

Dilute 10–20 cc of the milk with an equal volume of recently boiled and cooled H O and titrate with standard NaOH soln, using phenolphthalein indicator Express the result as percentage of lactic acid The determination may be conveniently made by measuring 17 6 cc of the prepared sample with the 17 6 cc Babcock pipet [18 (b)], diluting with an equal volume of recently boiled and cooled H_2O, washing out the pipet with CO_2 free H_2O, and titrating with 0 1 *N* NaOH soln, using 0 5 cc of phenolphthalein indicator The number of cc of 0 1 *N* NaOH soln required divided by 20 gives the percentage of lactic acid

5 TOTAL SOLIDS—OFFICIAL

Weigh a flat-bottomed dish of not less than 5 cm diameter If desired, the dish may have spread in it, prior to weighing, 15–20 g of pure dry sand Pipet into the dish 3–5 cc of the sample weigh quickly and heat at the temp of boiling H_2O until

it ceases to lose weight Cool in a desiccator and then weigh quickly to avoid the absorption of moisture Report the increase in weight as total solids

ASH—OFFICIAL

6 Into a weighed dish pipet about 20 cc of the prepared sample, weigh quickly add 6 cc of HNO_3, evaporate to dryness, and ignite at a temp below redness until the ash is free from C Cool in a desiccator, weigh and report the increase in weight as ash

TOTAL NITROGEN—OFFICIAL

7 Transfer 5 g of the sample to a Kjeldahl digestion flask and proceed as directed under II, 19, 22, or 24 Multiply the percentage of N by 6 38 to obtain the equivalent percentage of N compounds

CASEIN

(This determination should be made while the milk is fresh, or nearly so When it is not practicable to make this determination within 24 hours, add 1 part of HCHO to 2500 parts of milk and keep in a cool place)

Method I—Official

8 Place 10 g of the sample in a beaker with 90 cc of H_2O at 40–42°, and add at once 1 5 cc of acetic acid (1+9) Stir, and let stand 3–5 min Decant on a filter wash by decantation 2 or 3 times with cold H_2O, and transfer the precipitate to the filter Wash once or twice on the filter The filtrate should be clear, or very nearly so If the first portions of the filtrate are not clear, repeat the filtration and complete the washing of the precipitate Determine N in the washed precipitate and filter paper as directed under II, 19, 22 or 24, and multiply by 6 38 to obtain the equivalent of casein

To a sample of milk that has been preserved, the acetic acid should be added in small portions, a few drops at a time, with stirring, and the addition should be continued until the liquid above the precipitate becomes clear, or very nearly so

Method II—Tentative

REAGENT

Pipet 250 cc of N acetic acid into a 1000 cc flask Add 125 cc of normal CO_2-free NaOH Make up to 1000 cc with CO_2-free distilled H_2O and mix thoroly

DETERMINATION

9 Pipet 20 cc of the sample into a 100 cc flask Add 50 cc of the reagent, mix, make up to volume with distilled H_2O, and shake well Set the flask in hot H_2O (50–60 , not over 60) and let stand 15 min Cool to room temp , add 0 5 g of filter-cell, shake thoroly, and filter clear thru a suitable folded paper, taking care to prevent evaporation during filtration Determine N (A) in 50 cc of the clear filtrate, and determine total N (B) in 10 cc of the milk Multiply (B—A) by 6 38 This gives the casein in 10 cc of the milk Report grams of casein per 100 cc of milk, or divide the grams per 100 cc by the density of the milk and report as percentage by weight

ALBUMIN

Method I—Official

10 Exactly neutralize the filtrate obtained under 8 with 10% NaOH soln, add 0 3 cc of acetic acid (1+9) and heat on a steam bath until the albumin is completely pre

cipitated Collect the precipitate on a filter wash with cold H_2O, determine the N as directed under II, 19, 22 or 24, and multiply by 6 38 to obtain the equivalent of albumin

11 *Method II—Official**

LACTOSE

Optical Method—Official

12 REAGENTS

(a) *Acid mercuric nitrate soln* —Dissolve Hg in twice its weight of HNO_3 and dilute with an equal volume of H_2O

(b) *Mercuric iodide soln* —Dissolve 33 2 g of KI and 13 5 g of $HgCl_2$ in 20 cc of glacial acetic acid and 640 cc of H_2O

13 DETERMINATION

Determine the specific gravity of the milk as directed under 3 The quantity of sample to be taken for the determination varies with the specific gravity and is to be measured at the same temp at which the specific gravity is taken The volume to be measured will be found in the table under 14, which is based upon twice the normal weight of lactose (32 9 g per 100 cc) for the Ventzke sugar scale

Place the quantity of milk indicated under 14 in a flask graduated at 102 6 cc Add 1 cc of the acid $Hg(NO_3)_2$ soln or 30 cc of the HgI_2 soln (an excess of these reagents does no harm), fill to the mark, shake, filter thru a dry filter, and polarize It is not necessary to heat before polarizing If a 200 mm tube is used, divide the polariscope reading by 2 (or, if a 400 mm tube is used, by 4) to obtain the percentage of lactose in the sample

14 *Volumes of milk corresponding to a lactose double normal weight*[2]

SPECIFIC GRAVITY OF MILK	VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE)	SPECIFIC GRAVITY OF MILK	VOLUME OF MILK FOR A LACTOSE DOUBLE NORMAL WEIGHT (VENTZKE SCALE)
1 024	64 25	1 030	63 90
1 025	64 20	1 031	63 80
1 026	64 15	1 032	63 75
1 027	64 05	1 033	63 70
1 028	64 00	1 034	63 65
1 029	63 95	1 035	63 55
		1 036	63 50

15 *Gravimetric Method—Official*

Dilute 25 g of the sample with 400 cc of H_2O in a 500 cc volumetric flask and add 10 cc of $CuSO_4$ soln [XXXIV, 31 (a)] and about 7 5 cc of a KOH soln of such strength that 1 volume is just sufficient to precipitate completely the Cu as hydroxide from 1 volume of the $CuSO_4$ soln Instead of KOH soln of this strength 8 8 cc of 0 5 *N* NaOH soln may be used After the addition of the alkali soln the mixture must still have an acid reaction and contain Cu in soln Fill the flask to the 500 cc mark mix, filter thru a dry filter and determine lactose in an aliquot of the filtrate as directed under XXXIV, 54 or 56

* See note 7 p xvii

FAT

Roese-Gottlieb Method[a]—*Official*

16 Transfer 10 g of the sample to a Rohrig tube or a similar apparatus add 1 25 cc of NH_4OH (2 cc if the sample is sour), and mix thoroly Add 10 cc of 95% alcohol and mix well Add 25 cc of ether shake vigorously for 30 seconds, add 25 cc of petroleum ether (redistilled slowly at a temp below 65), and shake again for 30 seconds Let stand 20 min , or until the upper liquid is practically clear Draw off as much as possible of the ether fat soln (usually 0 5–0 8 cc will be left) into a flask thru a small quick acting filter Again extract the liquid remaining in the tube this time with 15 cc of each ether, shake vigorously 30 seconds after each addition, and allow to settle Draw off the clear soln thru the small filter into the same flask as before and wash the tip of the spigot, the funnel and the filter with a few cc of a mixture of the two ethers, in equal parts, free from suspended H_2O This third extraction yields less than 1 mg of fat, a third extraction is necessary This third extraction yields less than 1 mg of fat if the previous ether fat solns have been drawn off closely Add a glass bead and evaporate the ethers slowly on a steam bath, then dry the fat in a boiling H_2O oven to constant weight Weigh the flask with a similar flask as a counterpoise Do not wipe the flask immediately before weighing

Remove the fat completely with petroleum ether Deduct the weight of the dried flask with residue and bead to obtain weight of fat Finally, correct this weight by a blank determination on the reagents used

Babcock Method—*Official*

REAGENT

17 *Sulfuric acid* —Sp gr 1 82–1 83 at 20°

APPARATUS

18 (a) *Standard Babcock test bottle for milk* —S%, 18 g, 6 inch milk test bottle, total height 150–165 mm (5 9–6 5 inches) The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when the bottle stands on a level surface The charge of milk for the bottle shall be 18 g

Bulb —The capacity of the bulb to the junction with the neck shall be not less than 45 cc The shape of the bulb shall be either cylindrical or conical If cylindrical the outside diameter shall be between 34 and 36 mm, if conical the outside diameter of the base shall be between 31 and 33 mm and the maximum diameter between 35 and 37 mm

Neck —The neck shall be cylindrical and of uniform diameter from at least 5 mm below the lowest graduation mark to at least 5 mm above the highest The top of the neck shall be flared to a diameter of not less than 10 mm The graduated portion of the neck shall have a length of not less than 63 5 mm The total per cent graduation shall be 8 The graduations shall represent whole per cent, 0 5% and 0 1%, respectively, from 0 0 to 8 0% The tenths per cent graduations shall be not less than 3 mm in length the 0 5% graduations shall be not less than 4 mm in length and shall project 1 mm to the left, and the whole per cent graduations shall extend at least half way around the neck to the right and shall project at least 2 mm to the left of the tenths per cent graduations Each whole per cent graduation shall be numbered the number being placed to the left of the scale The capacity of the neck for each whole per cent on the scale shall be 0 20 cc The maximum error of the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation

Each bottle shall be so constructed as to withstand the stress to which it will be subjected in the centrifuge

(a_1) *Testing* —The Hg and cork, alcohol and buret, and alcohol and brass plunger methods may be employed for the rapid testing of the bottles, but the accuracy of any questionable bottle shall be determined by calibration with Hg (13 5471 g of clean, dry Hg at 20° to be equal to 5% on the scale of an 18-g bottle and 10% on the scale of a 9-g bottle), the bottle being previously filled to zero with Hg

(b) *Pipet* —The standard milk pipet shall conform to the following specifications

Total length not more than 330 mm
Outside diameter of suction tube 6–8 mm
Length of suction tube 130 mm
Outside diameter of delivery tube 4 5–5 5 mm
Length of delivery tube 100–120 mm
Distance of graduation mark above bulb 15–15 mm
Nozzle straight
Graduation to contain 17 6 cc of H_2O at 20°, when the bottom of the meniscus coincides with the mark on the suction tube

Delivery in 5–8 seconds
The maximum error in the graduation shall not exceed 0 05 cc
The pipet is to be marked "Holds 17 6 cc"

(b_1) *Testing* —The pipet shall be tested by measuring from a buret the volume of H_2O (at 20°) which it holds up to the graduation mark

(c) *Acid measure* —The device employed to measure H_2SO_4, whether a graduated cylinder or a pipet attached to a Swedish acid bottle, shall be graduated to deliver 17 5 cc

(d) *Centrifuge or "tester"*—The standard centrifuge, however driven, shall be constructed throuout and so mounted as to be capable, when filled to capacity, of rotating at the necessary speed with a minimum of vibration and without liability of causing injury or accident It shall be heated, electrically or otherwise, to a temp of at least 55° during the process of centrifugalizing It shall be provided with a speed indicator, permanently attached, if possible The proper rate of rotation may be ascertained by reference to the table below By "diameter of wheel" is meant the distance between the inside bottoms of opposite cups measured thru the center of rotation of the centrifuge wheel while the cups are horizontally extended

Diameter of wheel, in inches	10	12	14	16	18	20	22	24
No revolutions per minute	1074	980	909	848	800	759	724	693

(e) *Dividers or calipers* —For measuring the fat column

(f) *Water bath for test bottles* —Provided with a thermometer and a device for maintaining a temp of 55–60°

19 DETERMINATION

Transfer 18 g of the sample, prepared as directed under 2, to the milk test bottle by means of the pipet Blow out the milk remaining in the pipet tip after free out flow has ceased Add 17 5 cc of H_2SO_4 preferably not all at one time, pouring it down the side of the neck of the bottle in such a way as to wash any traces of the milk into the bulb The temp of the acid shall be about 15–20° Shake until all traces of curd have disappeared, then transfer the bottle to the centrifuge, counter balance it, and, after the proper speed has been attained, whirl 5 min Add soft H_2O at 60°, or above, until the bulb of the bottle is filled Whirl 2 min Add hot H_2O until the liquid column approaches the top graduation of the scale Whirl 1 min longer at a temp of 55–60° Tansfer the bottle to the warm water bath maintained at a temp of 55–60°, immerse it to the level of the top of the fat column, and leave it

there until the column is in equilibrium and the lower fat surface has assumed a final form Remove the bottle from the bath, wipe it and with the aid of dividers or calipers measure the fat column in terms of percentage by weight from its lower surface to the highest point of the upper meniscus

The fat column, at the time of measurement should be translucent of a golden yellow or amber color and free from visible suspended particles Reject all tests in which the fat column is milky or shows the presence of curd or of charred matter or in which the reading is indistinct or uncertain

ADDED WATER

I Acetic Serum Method Official

20 (a) *Cross immersion refractometer reading* —To 100 cc of the milk measured at 20° into a beaker, add 2 cc of 2 5% acetic acid (sp gr 1 035) Cover the beaker with a watch glass and place in a water bath at 70° for 20 min Place the beaker in ice H₂O for 10 min and separate the curd from the serum by rapid filtration thru a small filter Transfer a portion of the clear serum to a refractometer beaker place in the constant temp bath and take the refractometer reading when the temp of the serum has been brought to exactly 20° as determined by a thermometer graduated in tenths of a degree A reading below 39 indicates added H_2O between 39 and 40 the addition of H_2O is suspected When the reading is 40 or below determine the ash in the serum as directed under (b)

(b) *Ash* —Transfer 25 cc of the serum to a weighed flat bottomed Pt dish and evaporate to dryness on a water bath Heat over a low flame (to avoid spattering) until the contents are thoroly charred place the dish in an electric muffle preferably with pyrometer attached and ignite to a white ash at a temp not greater than 500° Cool and weigh Express the result as grams per 100 cc A result below 0 71 g per 100 cc indicates added H_2O The acetic serum ash multiplied by the factor 1 021 equals the sour serum ash (dilution of the acetic serum being 2%)

II Sour Serum Method —Official

21 (a) *Cross immersion refractometer reading* Allow the milk to become sour Heat, filter and determine the immersion refractometer reading of the clear serum at 20° A reading below 38 3 indicates added H_2O

(b) *Ash* Determine the ash in 25 cc of the serum obtained in (a) as directed under 20 (b) A result below 0 720 g per 100 cc indicates added H_2O

III Copper Serum Method —Official

22 To 1 volume of $CuSO_4 \cdot 5H_2O$ (2 5 g of $CuSO_4 \cdot 5H_2O$ per liter adjusted if necessary to read 1° at 20° on the scale of the Zeiss immersion refractometer or to a specific gravity of 1 0113 at 20°) add 14 volumes of milk Shake well and filter Determine the refractometer reading of the clear serum at 20° A reading below 36 indicates added H_2O When the refractometer reading is 37 or below determine the ash of the serum as directed under 21 (b) 1 cc of the acetic serum as directed under 20 (b)

IV Creamometer Method —Official

Apparatus

23 (a) *Creamometer* A glass cylinder 1 liter capacity and 4 25 cm in diameter [illegible] Dewar flask of 1 liter capacity and 9 25 cm in diameter [illegible] a large

cork of about 3 cm thickness Thru the center of the cork is tightly fitted a medium thin walled glass or metal tube, 250 mm in length by 33 mm outside diameter At one side of the cork is inserted a narrow metal inlet tube, the lower end of which is formed into a perforated loop near the bottom of the flask At the opposite side is a metal tube of T-shape construction and 6 mm internal diameter, intended to afford escape for vapors, and also for introducing volatile fluid into the apparatus At the back portion of the cork is fitted a control thermometer, the bulb of which extends nearly to the bottom of the flask The freezing test tube is of thin glass, about 240 mm in length by 29 mm outside diameter, and fits closely into the larger tube, which is sealed into the cork In the rubber stopper of the freezing tube is fitted the standard thermometer The length of the thermometer permits insertion of the bulb nearly to the bottom of the tube and at the same time allows complete exposure of the scale above the stopper At the right side of the thermometer a stirring device made of noncorrodible low conductivity metal is fitted into the stopper thru a short section of thin walled metal tubing The lower end extends nearly to the bottom of the test tube and is provided with a horizontal loop encircling the thermometer At the left of the thermometer is a freezing starter attachment inserted thru an opening in the stopper formed by means of a short section of metal tubing This device consists of a non corrodible metal rod, at the lower end of which is a 10 mm length opening for the purpose of carrying a small fragment of ice At one side of the cryoscope is installed an air drying arrangement which consists of a Folin absorption bulb inserted thru a tightly fitting stopper and extending nearly to the bottom of a large sized test tube A short section of glass tubing is inserted thru a second opening in the stopper and is connected to the vaporizing tube which enters the cryoscope Sulfuric acid is poured into the drying tube to a level slightly above the small inner bulb At the opposite side of the apparatus is arranged a drain tube for the purpose of conducting vapors away from the operator By means of a pressure and suction pump dry air may be forced into the apparatus at a suitable rate and the mixed vapors conducted out thru the base of the drain tube into the sink An adjustable lens is mounted in a convenient position in front of the thermometer for the purpose of magnifying the scale

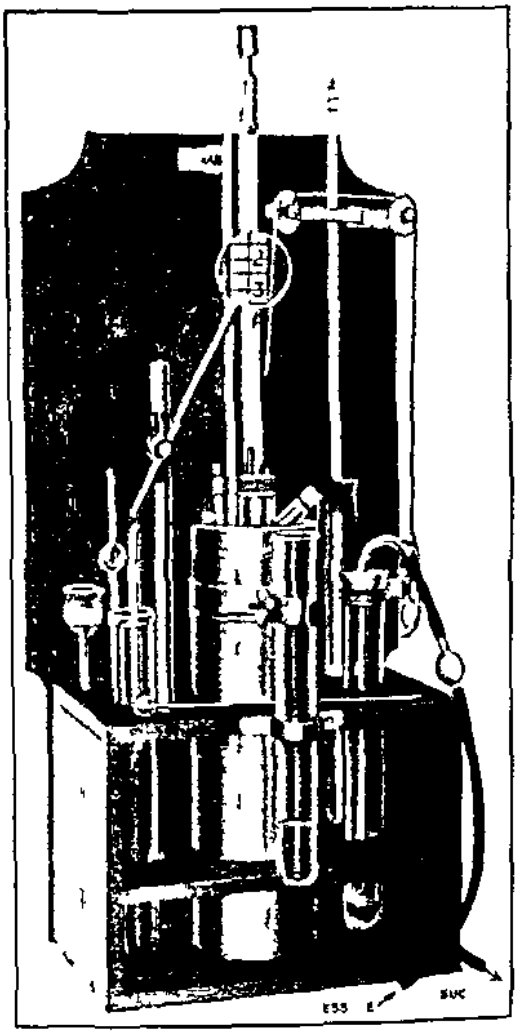

FIG 22—HORTVET CRYOSCOPE

(b) *Standard thermometer*—A solid stem instrument having a total length of 58 cm, with a scale portion measuring about 30 cm The total scale range is 3°, from +1° to −2°, and each degree division is subdivided into tenths and hundredths The length of a degree division approximates 1 dm, thus making the smallest subdivisions of such magnitudes as to enable easy observation and readings estimated

to 0 001° Standardize the thermometer as directed under 24 Check at frequent intervals, once a week or as often as may be necessary, to keep an accurate record of any changes that may occur

(c) *Control thermometer* —A solid stem instrument approximately 58 cm in length and having a scale range of +20° to −30° Test in a bath of melting crushed ice for the purpose of determining whether the 0 mark on the scale is correct The scale graduations should be accurate to within 0 10

STANDARDIZATION OF THE THERMOMETER

24 Make 3 freezing-point determinations by the procedure given under 25 on each of the following

(a) *Recently boiled distilled water*

(b) *Sucrose soln* —Dissolve 7 g of pure sucrose in H_2O and make the soln to a volume of 100 cc at 20°

(c) *Sucrose soln* —Dissolve 10 g of pure sucrose in H_2O and make the soln to a volume of 100 cc at 20°

(A sample of pure sucrose may be obtained by application to the Director of the Bureau of Standards, Department of Commerce, Washington, D C)

Tabulate the results in the following form

FREEZING POINT OBSERVATIONS	PURE WATER	7 GRAMS SUCROSE SOLUTION		10 GRAMS SUCROSE SOLUTION	
		Observed freezing point (−b)	Freezing point depression S—W (algebraic)	Observed freezing point (−S)	Freezing point depression S—W (algebraic)
1st					
2nd					
3rd				YYXXXYY	
Averages	±W	XXXXYXX			

Express the results as degrees freezing point depression below the average of the observed freezing points obtained on the sample of pure H_2O (±W), which may be above (+) or below (−) the 0 mark on the scale Obtain each freezing point depression of the sucrose solns by the algebraic subtraction of the average of the freezing point readings of pure H_2O (±W) from each observed freezing point

Omit adventitious results, i e , results which are in marked disagreement with other results obtained by carefully following instructions

Apply the average of the freezing point depressions obtained on the standard sucrose solutions for the purpose of correcting the thermometer readings obtained on samples of milk in the manner illustrated in the tables accompanying Fig 23

DETERMINATION

25 (Make freezing point determinations only on samples of milk that show an acidity of not more than 0 18% when determined as directed under 4)

Insert the funnel tube into the vertical portion of the T tube at one side of the apparatus and pour in 400 cc of ether previously cooled to 10 or lower Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air drying attachment Adjust the pump so as to pass air thru the apparatus at a moderate rate, as may be judged by the agitation of the H_2SO_4 in the drying tube Continuous vaporization of the ether will cause a lowering of the temp

in the flask, from ordinary room temp to 0° in from 5 to 10 min Continue the temp lowering until the control thermometer registers near −3° At this stage, by lowering the gage tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made as to the quantity of ether necessary to pour in for the purpose of restoring the 100 cc volume When the volume of ether has been adjusted to 100 cc an additional 10–15 cc is sufficient on an average for each succeeding determination Pour into the freezing test tube sufficient H O (30–35 cc) boiled and cooled to 10° or lower, to fairly submerge the thermometer bulb Insert the thermometer together with the stirrer and lower the test tube

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Two Bureau of Standards tested thermometers give intervals of 0 199° and 0 200°, respectively, between the freezing-point depression readings of the two sucrose solns One thermometer gives freezing point depressions −0 422° and −0 622°, respectively for the two sucrose solns, while the other gives −0 422° and −0 621°, respectively

Laboratory Thermometer No 2

WATER	7 GRAMS SUCROSE TO 100 cc	10 GRAMS SUCROSE TO 100 cc
+0 056	−0 425°	−0 621°

Interval = 0 196
0 196 equiv 0 199
Correction = ×1 015

WATER
+0.079

Laboratory Thermometer No 24

WATER	7 GRAMS SUCROSE TO 100 cc	10 GRAMS SUCROSE TO 100 cc
0 000	−0 420°	−0 625°

INTERVAL
0199°

7 m SUCROSE 100 cc.
DEPRESSION −0 422°

10 m SUCROSE 100 cc
DEPRESSION −0621

Interval = 0 205
0 205 equiv 0 199
Correction = ×0 971

Example

Laboratory Thermometer No
F pt Depression Sample Milk
(0 548 − 0 420) 0 971 = 0 124
Corrected Depression = 0 422 + 0
= 0 546

3 — U S
DARD
ERMO

into the larger tube A small quantity of alcohol, sufficient to fill the lower space between the 2 test tubes, will serve to complete the conduction medium between the freezing bath and the liquid to be tested Keep the stirrer in steady up and down motion at a rate of approximately one stroke each 1 or 2 seconds, or even at a slower rate, providing the cooling proceeds satisfactorily Maintain a passage of air thru the apparatus until the temp of the cooling bath reaches −2 5 , at which time the top of the Hg thread in the thermometer usually recedes to a position near the freezing point of H_2O Maintain the temp of the cooling bath at −2 5 and continue the manipulation of the stirrer until a super cooling of sample of 1 0 to 1 2° is observed As a rule, at this time the liquid will begin to freeze, as may be noted by the rapid rise of the Hg Manipulate the stirrer slowly and carefully 3 or 4 times as the Hg column approaches its highest point By means of a suitable light weight cork mallet tap the upper end of the thermometer cautiously a number of times until the top of the Hg column remains stationary for at least 1 min Observe the exact reading on the thermometer scale, taking necessary precautions to avoid parallax and estimate to 0 001 When the observation has been satisfactorily completed, make a duplicate determination, then remove the thermometer and stirrer and empty the H_2O from the freezing tube

Rinse the tube with about 25 cc of the sample of milk, cooled to 10° or lower, measure into the tube 30–35 cc of milk or enough to fairly submerge the thermometer bulb, and insert the tube into the apparatus Maintain the temp of the cooling bath at 2 5° below the probable freezing point of the sample Make the determination on the milk, following the same procedure as that employed in determining the freezing point of H_2O As a rule, however it is necessary to start the freezing action in the milk by inserting the freezing starter (which has been kept in contact with ice for several minutes and in the open end of which has been wedged a fragment of ice) at the time when the Hg column has receded to 1 0–1 2 below the probable freezing point A rapid rise of the Hg results almost immediately Manipulate the stirrer slowly and carefully 2 or 3 times while the Hg approaches its highest point Complete the adjustment of the Hg column in the same manner as in the preceding determination, then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to 0 001° *The algebraic difference* between the average of readings obtained on the H_2O and the reading obtained on the sample of milk represents the *freezing point depression* of the milk Apply necessary correction to the result in the manner shown in the illustrative tables accompanying Fig 23

Ascertain the percentage of added H_2O corresponding to the determined freezing point depression from Table 23 under XLII The percentage of added H_2O (W) may also be calculated as follows

$$W = \frac{100\,(T - T_1)}{T}, \text{ in which}$$

T = the average freezing point of normal milk (−0 550) and

T_1 = the observed freezing point on a given sample

A tolerance of 3% may be allowed on results for added H_2O determined on the basis of an average freezing point depression of −0 530° Owing to the narrow variations found in market milk of genuine character it is not necessary to deduct the tolerance figure from results showing added H_2O in excess of 3%

GELATIN

Qualitative Test—Official

26

To 10 cc of the milk add an equal volume of acid $Hg(NO_3)_2$ soln (Hg dissolved in twice its weight of HNO_3 and this soln diluted to 25 times its volume with H_2O)

overheating the sample, thereby causing the cream to "oil off" This precaution is especially necessary in the case of a thin cream

TOTAL SOLIDS—OFFICIAL

31 Proceed as directed under 5, using 2–3 g of the sample

ADDED WATER IN CREAM—OFFICIAL FIRST ACTION

32 Proceed as directed under 23, but use the following formula to calculate the percentage of added H_2O

$$W = \frac{\%\ Serum\ in\ Cream\ (T - T')}{T}$$ in which

W = the percentage of added H_2O,
T = the freezing point of undiluted cream ($-0.550°$),
T' = the observed freezing point of the given sample, and
$\%\ Serum = 100\% - (\%\ fat + \%\ protein)$

If protein is not determined it may be assumed to be 38% of the solids not fat

ASH—OFFICIAL

33 Proceed as directed under 6

TOTAL NITROGEN—OFFICIAL

34 Proceed as directed under 7

LACTOSE

Gravimetric Method—Official

35 Proceed as directed under 15

FAT

Roese Gottlieb Method—Official

36 Transfer 5 g of the sample to a Röhrig tube or a similar apparatus dilute with H_2O to about 10 5 cc, and proceed as directed under 16

Babcock Method—Official

REAGENTS

37 (a) *Sulfuric acid*—Sp gr 1 82–1 83 at 20
(b) *Glymol, or clear white mineral oil*—Sp gr not to exceed 0 85 at 20° Oil soluble artificial color may be added to the oil

APPARATUS

38 (a) *Test bottles*—The standard Babcock test bottles for cream shall be as follows (1) 50%, 6 g, *short necked, 6 inch cream test bottle*—Total height 150–165 mm (5 9–6 5 inches) The bottom of the bottle shall be flat and the axis of the neck shall be vertical when the bottle stands on a level surface The charge of cream for the bottle shall be 9 g

Bulb—The capacity of the bulb to the junction with the neck shall be not less than 45 cc The shape of the bulb shall be either cylindrical or conical If cylindrical, the outside diameter shall be between 34 and 36 mm, if conical the outside diameter of the base shall be between 31 and 33 mm, and the maximum diameter between 35 and 37 mm

Neck—The neck shall be cylindrical and of uniform diameter from at least 5 mm below the lowest graduation mark to at least 5 mm above the highest The top of

the neck shall be flared to a diameter of not less than 15 mm The graduated portion of the neck shall have a length of not less than 63 5 mm The total per cent gradua tion shall be 50 The graduations shall represent 5%, 1%, and ½%, respectively, from 0 0 to 50% The 5% graduations shall extend at least half-way around the neck to the right, the ½% graduations shall be not less than 3 mm in length, and the 1% graduations shall be intermediate in length between the 5% and ½% gradua tions and shall project 2 mm to the left of the ½% graduations Each 5% gradua tion shall be numbered (thus 0, 5, 10, 15, 50), the number being placed to the left of the scale The capacity of the neck for each whole per cent on the scale shall be 0 1 cc The maximum error in the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation

(2) *50%, 9 g, long necked, 9 inch cream test bottle* —The same specifications shall apply to this bottle as to the 50%, 9 g, 6 inch cream test bottle, except that the total height of this bottle shall be 210–220 mm (8 25–9 0 inches) and the gradu ated portion of the neck shall have a length of not less than 120 mm

(3) *50%, 18 g, long necked, 9 inch cream test bottle* —The same specifications shall apply to this bottle as to the 50%, 9 g, 9 inch cream test bottle, except that the charge of cream for this bottle shall be 18 g

Each bottle shall bear on the top of the neck above the graduations, in plain legi ble characters, a mark denoting the weight of the charge to be used, *viz*, "9 g" or "18 g," as the case may be

Each bottle shall be so constructed as to withstand the stress to which it will be subjected in the centrifuge

(4) *Testing* —Proceed as directed under 18(a_1)

(b) *Water bath for cream samples* —Provided with a thermometer and a device for maintaining a temp of 38–50°

(c) *Cream weighing scales* —With a sensibility reciproc al of 30 mg, *i e*, the addi tion of 30 mg to either pan of the scale, when loaded to capacity, shall cause a de flection of at least 1 subdivision of the graduation The scales shall be set level upon a table support and be protected from drafts

(d) *Weights* —9 g and 18 g, respectively, and plainly marked "9 g" or "18 g," as the case may be They shall be made of material capable of resisting corrosion or other injury, shall preferably be of a low squat shape, with rounded edges, and shall be verified at frequent intervals by comparison with standardized weights

(e) *Acid measure* —Described under 18(c)

(f) *Centrifuge or "tester"* —Described under 18(d)

(g) *Dividers or calipers* —Described under 18(e)

(h) *Water bath for test bottles* —Described under 18(f)

39

DETERMINATION

Weigh 9 g of the sample prepared as directed under 30, directly into a 9 g cream test bottle or 18 g into an 18-g bottle, and proceed by one of the following methods

Method 1—After the cream has been weighed into the test bottle add 8–12 cc of the H_2SO_4 in the case of the 9 g bottle, or 14–17 cc of the acid in the case of the 18 g bottle, or add acid until the mixture of cream and acid, after shaking has assumed a chocolate brown color Shake until all lumps have completely disappeared then add 5–10 cc of soft H_2O at 60 or above Transfer the bottle to the centrifuge, counterbalance it, and, after the proper speed has been attained, whirl 5 min Add hot H_2O until the liquid column approaches the top graduation of the scale, then whirl 1 min longer at a temp of 55–60 Adjust the temp, as directed under 19, and, with the aid of dividers or calipers, measure the fat column, in terms of percent age by weight, from its lower surface to the bottom of the upper meniscus

Method 2.—For a 9 g bottle only.—After the cream has been weighed into the test bottle add 9 cc of soft H_2O and thoroly mix, add 17 5 cc of the H_2SO_4 and shake until all lumps have completely disappeared Transfer the bottle to the centrifuge, counterbalance it, and, after the proper speed has been attained, whirl 5 min Fill the bottle to the neck with hot H_2O and whirl 2 min Add hot H_2O until the liquid column approaches the top graduation of the scale, then whirl 1 min longer at a temp of 55–60 Adjust the temp and measure the fat column as directed under *Method 1*

Whichever method is followed, the fat column at the time of reading, should be translucent, of a golden yellow to amber color, and free from visible suspended particles All tests in which the fat column is milky or shows the presence of curd or of charred matter or in which the reading is indistinct or uncertain should be rejected

If glymol is used a few drops only should be introduced into the bottle just before the reading is made, it must not be dropped in, but must be allowed to flow down the side of the neck For the purpose of measurement, the surface separating the glymol and the fat is regarded as representing the upper limit of the column

GELATIN—OFFICIAL

40 Proceed as directed under 26

PRESERVATIVES—OFFICIAL

41 Proceed as directed under 27 and under XXXII

COLORING MATTERS—OFFICIAL

42 Proceed as directed under 28 and under XXI

EVAPORATED MILK (UNSWEETENED)

PREPARATION OF SAMPLE—OFFICIAL

43 (a) Transfer the entire contents of a can to a large dish stir thoroly and pass thru a fine sieve or strainer until a homogeneous mass is secured If a slight separation of fat is evident, warm a portion of the sample containing the separated fat to 30–35° and agitate until a uniform emulsion is obtained, then combine with the unheated portion and mix thoroly (If an appreciable quantity of fat has separated rendering impossible the formation of a satisfactory emulsion an accurate analysis cannot be made)

(b) Dilute 40 g of the homogeneous mass prepared as directed under (a) with 60 g of H_2O and mix thoroly

TOTAL SOLIDS—OFFICIAL

44 Weigh 4–5 g of the diluted sample 43(b), into a weighed flat bottomed Pt dish not less than 5 cm in diameter and proceed as directed under 5 Correct the result for the dilution

ASH—OFFICIAL

45 Ignite the residue from the total solids determination 44, at a low red heat until the ash is free from C Correct the result for the dilution

FAT—OFFICIAL

46 Weigh 4–5 g of the undiluted sample, 43(a) into a Rohrig tube or a similar apparatus dilute with H_2O to about 10 5 cc, and proceed as directed under 16

TOTAL NITROGEN—OFFICIAL

47 Weigh 5 g of the undiluted sample 43(a), transfer to a Kjeldahl flask and proceed as directed under 11, 19, 22 or 24 Multiply the percentage of N by 6 38 to obtain the equivalent percentage of N compounds

48 CASEIN—OFFICIAL

Weigh 10 g of the diluted sample, 43(b), into a beaker, and proceed as directed under 8 or 9 Correct the result for the dilution

49 ALBUMIN—OFFICIAL

Proceed as directed under 10, using the filtrate from 48 Correct the result for the dilution

50 LACTOSE—OFFICIAL

Proceed as directed under 13 or 15, using the diluted sample, 43(b), and correct the result for the dilution

51 GELATIN—OFFICIAL

Proceed as directed under 26

52 PRESERVATIVES—OFFICIAL

Proceed as directed under 27 and under XXXII

53 COLORING MATTERS—OFFICIAL

Proceed as directed under 28 and under XXI

SWEETENED CONDENSED MILK

54 PREPARATION OF SAMPLE—OFFICIAL

(a) If the can is cold, place it in H_2O at 30–35° until warm Open, scrape out all milk adhering to the interior of the can, and after transferring to a dish sufficiently large to permit stirring thoroly mix until the whole mass is homogeneous

(b) Weigh 100 g of the thoroly mixed sample into a 500 cc volumetric flask, dilute to the mark with H_2O, and mix thoroly If the sample will not emulsify uniformly, weigh out a separate portion of (a) for each determination

55 TOTAL SOLIDS—OFFICIAL

Use 10 cc of the soln, prepared as directed under 54(b), and proceed as directed under 5, drying on either sand or asbestos fiber Correct the result for the dilution

56 ASH—OFFICIAL

Evaporate 10 cc of the soln, prepared as directed under 54(b), to dryness on a water bath and ignite the residue as directed under XXVII, 8 Correct the result for the dilution

57 PROTEIN—OFFICIAL

Determine the N as directed under II, 19, 22, or 24, using 10 cc of the soln prepared as directed under 54(b), and multiply by 6 38 to obtain the equivalent of protein Correct the result for the dilution

58 LACTOSE—OFFICIAL

Dilute 100 cc of the soln, prepared as directed under 54(b), in a 250 cc volumetric flask to about 200 cc, add 6 cc of Fehling s $CuSO_4$ soln (XXXIV, 31(a)), make up to the mark, and mix thoroly Filter thru a dry filter and determine lactose as directed under XXXIV, 54 or 56 Correct the result for the dilution

59 FAT—OFFICIAL

Weigh accurately 4–5 g of the homogeneous sample, prepared as directed under 54(a), into a Röhrig tube or a similar apparatus, dilute with H_2O to about 10 5 cc, and proceed as directed under 16

SUCROSE—OFFICIAL

REAGENT

60 To 220 g of yellow HgO, add 300–400 cc of H_2O and sufficient HNO_3 to form a clear soln (about 110 cc), being careful to use the least possible excess of acid Dilute to 800–900 cc and add 10% NaOH soln slowly and with constant shaking until a slight permanent precipitate is obtained Dilute to 1 liter and filter The soln tends to become acid with age owing to the deposition of basic mercuric salts For this reason dilute alkali should be added occasionally until a slight permanent precipitate is formed and the soln filtered

DETERMINATION

61 Introduce 50 cc of the soln, prepared as directed under 54(b), into a 100 cc volumetric flask add 25 cc of H_2O, mix, add 5 cc of the $Hg(NO_3)_2$ reagent, and shake thoroly Without delay and while shaking constantly, add sufficient 0 5 *N* NaOH soln to render the mixture neutral to litmus paper, being careful to avoid an alkaline reaction (usually 12–13 cc will be required) Dilute to 100 cc with H_2O, mix thoroly, and filter thru a dry paper Polarize the filtrate in a 200 mm tube, then invert at room temp as directed under XXXIV, 23(c), and polarize the inverted soln Correct both readings for the volume occupied by the protein, 57, and the fat, 59, 1 g of protein occupying a space of 0 8 cc and 1 g of fat, 1 075 cc Calculate the percentage of sucrose by the following formula, using the corrected direct and invert readings obtained above

$$S = \frac{100\,(a-b)}{142\ 35 - \frac{t}{2}} \times \frac{26}{W}, \text{ in which}$$

S = percentage of sucrose in the sample,
a = corrected direct polarization,
b = corrected invert polarization,
t = temp of soln polarized, and
W = weight of sample taken (10 g)

DRIED MILK AND MALTED MILK

PREPARATION OF SAMPLE—TENTATIVE

62 Sift the sample thru a 20 mesh sieve onto a large sheet of paper rubbing the material thru the sieve and tapping vigorously if necessary Grind the residue in a mortar pass thru the sieve and mix into the sifted material Discard particles of wood and other material which cannot be ground Sift the sample 2 more times mixing thoroly each time To avoid absorption of moisture operate as rapidly as possible and preserve the sample in an air tight container

MOISTURE—TENTATIVE

APPARATUS

63 *Metal dish*.—Diameter about 55 mm, height about 15 mm, provided with a slip in inverted cover fitting tightly on the inside

DETERMINATION

64 Weigh 1–1 5 g of the sample into the previously weighed metal dish cover tightly, and reweigh Dry in the loosely covered dish placed in direct contact with the metal shelf of the vacuum oven to constant weight (approximately 5 hours) under a pressure not to exceed 100 mm (4 inches) of Hg at the temp of boiling H_2O During the

drying admit into the oven a slow current of air (about 2 bubbles per second), dried by passing thru H_2SO_4 Discontinue the action of the vacuum pump and carefully admit dried air into the oven Press the cover tightly into the dish, remove the dish from the oven, cool, and weigh Calculate the percentage loss in weight as moisture

65 PROTEIN—TENTATIVE

Weigh 1 g of the sample into a Kjeldahl digestion flask and determine N as directed under II, 24 Multiply the N by 6 38 to obtain the equivalent of N compounds

66 ASH—TENTATIVE

Ignite 1 g of the sample at a low red heat until free from C Cool in a desiccator and weigh

67 FAT (FOR MALTED MILK)[11]—TENTATIVE

Weigh accurately about 1 g of the well mixed sample into a small, lipped beaker Add 1 cc of H_2O and mix well with a glass rod to form a thick liquid free from lumps Add 10 cc more of H_2O warm on the steam bath, and transfer to a Röhrig tube or similar apparatus Cool, add 10 cc of 95% (by volume) alcohol, and mix Add 25 cc of ethyl ether and proceed with the extraction as in the official Roese-Gottlieb method for milk, 16 Dissolve the dried fat in petroleum ether and determine the quantity of any insoluble residue that may be present

68 FAT (FOR DRIED MILK)[11]—TENTATIVE

Proceed as under 67 Add after the first extraction with ether 4 cc of 95% alcohol to the liquid remaining in the extraction apparatus, mix, and proceed with the second extraction If necessary, in the third extraction add sufficient H_2O to raise the level of the aqueous layer to its original volume With whole milk and cream powders make a third extraction, using 15 cc of each ether

69 ROESE-GOTTLIEB METHOD (FOR DRIED MILK)[11]—TENTATIVE

Weigh out about 1 g of well mixed sample into a small, lipped beaker Add about 1 cc of H_2O and mix well with a glass rod to form a thick liquid free from lumps Add 9 cc more of H_2O and 1 cc of NH_4OH, warm on the steam bath, and transfer to a Rohrig tube or similar apparatus Cool, add 10 cc of 95% (by volume) alcohol and mix Add 25 cc of ethyl ether, shake vigorously for 30 seconds, and proceed as directed under 16 For the second extraction add 4 cc of 95% alcohol, extract the liquid remaining in the tube, and again proceed as directed under 16 Make the third extraction, if necessary, like the second adding sufficient H_2O to raise the aqueous layer to its original volume Dissolve the dried fat in petroleum ether and determine the quantity of any insoluble residue that may be present In case of whole milk and cream powders, make a third extraction, using 15 cc of each ether, and proceed as under 16

70 MICROSCOPICAL IDENTIFICATION OF MALTED MILK AND ITS FLAVORED PRODUCTS[11]—TENTATIVE

Mount a small quantity of the material in a drop of mineral oil on a slide, apply the cover glass and examine the preparation at a magnification of approximately 200 using a microscope lamp with daylight glass as a source of light Control the light intensity by the iris diaphragm because a too brilliantly lighted field hinders the recognition of details (See pp 232-235)

BUTTER

(The following methods are also applicable to renovated or process butter and margarine)

72 DIRECTIONS FOR SAMPLING—OFFICIAL*

73 SAMPLING—OFFICIAL (FIRST ACTION)

(a) *Tub butter*—Insert an ordinary trough butter trier for practically its full length vertically thru the butter mass at a point approximately half way between the center and the edge of the tub Give the trier one complete turn and withdraw a full core Transfer the core immediately to the sample container with the aid of a spatula Do not include moisture adhering to the outside of the trier When more than 1 core is taken from a tub, withdraw the cores at different points around the circumference of an imaginary circle half way between the center and edge of the tub Wipe the trier clean and dry after each core is removed Fill the trier holes with a plug of butter approximately 1 inch long procured from an extra core For butter stored at a moderate temp use an unwarmed trier, for very hard butter use a warmed trier Frozen butter should be stored 24 hours in a tempering room before sampling

Sample as a minimum, in the case of—

(1) Tubs marked with churn batch numbers, 3 tubs from each batch in the lot Take 1 core from each of 3 tubs in the batch Composite the 3 full cores of each batch separately

(2) Tubs not marked with churn numbers, the number of tubs indicated in the schedule below Take for each sub sample 3 cores from each tub

(b) *Print butter*—Withdraw as a minimum sub samples of at least ¼ pound each according to the schedule Remove the wrapper and place each sub sample in a separate sample container If print butter is identified by batch number, sample as under tub (a), except to analyze the 3 sub samples separately

Sampling Schedule

NO OF UNITS (TUBS OR CASES) IN THE LOT	NO OF UNITS TO BE SAMPLED
1–10	3
11–25	5
26–50	6
51–75	8
76–100	10

Take a greater number of sub samples from tub or print butter if circumstances indicate the necessity

(c) *Sample containers*—Use a glass jar, preferably with glass top of such type as will prevent loss of moisture by evaporation or entrance of H_2O into the jar Tops containing a liner of any material should not be used

74 PREPARATION OF SAMPLE—OFFICIAL

Soften the entire sample in a closed vessel at as low a temp as possible Shake vigorously until a perfectly homogeneous semi solid mass is obtained Weigh the portions for analysis at once If the sample is kept for any length of time it must be softened and shaken until semi solid before portions are withdrawn for analysis

75 *Mechanical Stirrer Method*—*Tentative*

Soften the sample, 250–500 g in a closed vessel, to such an extent that on stirring for 2–3 min the product will reach a temp of 31–34° Stir with a motor driven

* See p 1 [illegible]

CASEIN ASH AND SALT—OFFICIAL

81 Cover the crucible containing the residue from the fat determination by the in-
direct method, 79, and heat, gently at first, then raise the temp gradually to just
below redness The cover may then be removed and heating continued until the con
tents of the crucible are white The loss in weight represents casein, and the residue
in the crucible mineral matter Dissolve this mineral matter in H_2O slightly acidi
fied with HNO_3 and determine Cl either gravimetrically as directed under XII, 33,
or volumetrically as directed under XII, 35, and calculate the NaCl

SALT—OFFICIAL

82 Weigh in a counterpoised beaker 5–10 g of the sample add about 20 cc of hot
H_2O and after the butter has melted transfer the whole to a separatory funnel In
sert the stopper and shake for a few moments Let stand until all the fat has col
lected on the top of the H_2O, then draw off the H_2O into a flask being careful to let
none of the fat globules pass Again add hot H O rinsing the beaker and repeat the
extraction 10 to 15 times using 10–20 cc of H_2O each time The washings will con-
tain all but a mere trace of the NaCl originally present in the butter Determine the
quantity in the whole or in an aliquot of the liquid by titration with standard $AgNO_3$
soln using K_2CrO_4 indicator

EXAMINATION OF FAT—OFFICIAL

83 Melt the butter and keep in a dry place at about 60° for 2–3 hours or until the
H_2O and curd have entirely separated Filter the clear, supernatant fat thru a dry
filter paper in a hot water funnel or in an oven at about 60 If the filtered liquid fat
is not perfectly clear, refilter Determine physical and chemical constants as directed
under XXXI

PRESERVATIVES—OFFICIAL

84 Proceed as directed under 27 and under XXXII

COLORING MATTERS—OFFICIAL

85 Pour about 2 g of the filtered fat dissolved in ether into each of 2 test tubes Into
one of the tubes pour 1–2 cc of HCl (1+2) and into the other about the same vol
ume of 10% NaOH soln Shake the tubes well and allow to stand In the presence
of azo dyes the test tube to which the acid has been added will show a pink to wine
red coloration, while the alkaline soln in the other tube will show no color If on the
other hand, annatto or other vegetable color is present the alkaline soln will be col
ored yellow while no color will be apparent in the acid soln

General test—Proceed as directed under XXI, particularly 3 and 21, for the de
tection of oil soluble coal tar dyes and annatto

MICROSCOPIC EXAMINATION—OFFICIAL

86 (a) Place on a slide a small portion of the fresh, unmelted sample taken from the
inside of the mass add a drop of pure olive oil apply a cover glass with gentle pres
sure and examine with a magnification of 120–150 diameters for crystals of lard
etc Examine another portion of the sample with polarized light and selenite plate
without the use of oil Pure fresh butter will show neither crystals nor a parti colored
field with selenite Renovated butter or other fats melted and cooled and mixed with
butter will usually present crystals and variegated colors with the selenite plate

(b) For further microscopic study dissolve in a test tube 3–4 cc of the fat in 15 cc
of ether Close the tube with a loose plug of cotton wool and allow to stand 12–24
hours at 0–2 When crystals form at the bottom of the tube remove with a pipet

let the mixture separate Proceed from this point as directed under 16, beginning with "Draw off as much as possible of the ether fat soln"

99 EXAMINATION OF FAT—OFFICIAL

(a) *Alkaline extraction* —Treat about 300 g of the cheese, cut into fragments the size of a pea, with 700 cc of 5% KOH soln at 20° in a large, wide necked flask shaking vigorously to dissolve the casein In 5–10 min the casein will be dissolved, and the fat will rise to the surface in lumps Collect the lumps of fat into as large a mass as possible by shaking gently Pour cold H_2O into the flask until the fat is driven up into the neck and remove it by suitable means Wash the fat thus obtained with just sufficient H_2O to remove the residue of the alkali which it may contain The fat is not perceptibly attacked by the alkali in this treatment, is practically all separated in a short time, and is then easily prepared for chemical analysis by filtering and drying as directed under 83 Examine the fat as directed under XXXI

(b) *Acid extraction* —Pass the cheese thru a grinding machine, transfer to a large flask, and cover with warm H_2O, using 1 cc for every gram of cheese Shake thoroly and add H_2SO_4 slowly and in small quantities, shaking after each addition of acid The total quantity of acid used should be the same as the quantity of H_2O employed Remove the fat, which separates after standing a few min, by means of a separatory funnel, wash free from acid, filter, and dry as directed under 83 Examine the fat as directed under XXXI

TARTARIC ACID[a]

100 *Qualitative Test—Tentative*

To 5 g of the ground cheese, add 40 cc of H_2O at a temp of about 50° and shake until the cheese is thoroly broken up Add 3 cc of a 1% H_2SO_4 soln and shake vigorously then add 2 cc of a 20% soln of phosphotungstic acid and again shake vigorously Let stand for 5 min and filter To 25 cc of the filtrate add sufficient saturated Ba(OH) soln to make alkaline and 25 cc of 95% (by volume) alcohol, shake vigorously, and allow to settle Filter thru a Buchner funnel, using light suction, and wash the residue on the filter several times with H_2O Transfer a portion of the paste to a small evaporating dish and dry on the steam bath Add a few cc of concentrated H_2SO_4 and a few crystals of resorcin, and heat slowly If tartaric acid is present there is produced a rose red color that is slowly discharged on dilution with H O

Quantitative Method—Tentative

101 REAGENTS

(a) *Hydrochloric acid* —Approximately 2% soln Dilute 47 cc of HCl to 1 liter with H_2O

(b) *Sodium oxalate soln* —Dissolve 2 g of Na-oxalate in 100 cc of H_2O

(c) *Potassium chloride wash soln* —Dissolve 15 g of KCl in 100 cc of H_2O and add 20 cc of 95% (by volume) alcohol

(d) *Tartaric acid soln* —Dissolve 1 5 g of pure tartaric acid in previously boiled and cooled H_2O and dilute to 100 cc at 20° Titrate with 0 1 *N* NaOH soln to determine the quantity of tartaric acid in 10 cc of the soln

(e) *Potassium chloride* —Finely powdered

102 DETERMINATION

Weigh 25 g of the ground cheese into a 500 cc wide mouthed salt bottle and add, 25 cc at a time, 100 cc of H_2O at a temp of 50–60° shaking vigorously after each

addition If necessary, continue the shaking until the cheese is thoroly broken up Then add 25 cc of the Na-oxalate soln and shake vigorously for 1 min Add 100 cc of the HCl soln, 25 cc at a time, shaking vigorously after each addition Add 50 g of the powdered KCl and shake for 5 min To avoid churning keep the mixture warm (at about 50°) during the shaking Transfer the contents of the bottle, with the aid of H_2O, to a 300 cc volumetric flask, cool to 20°, and make up to the mark with H_2O Mix thoroly let stand for 10 min with occasional shaking, and then filter thru a dry folded filter, discarding the first few cc of the filtrate Disregard any opalescence and transfer 200 cc of the filtrate to a 250 cc volumetric flask Neutralize with 1 *N* NaOH soln using phenolphthalein indicator, and then add 5 2 cc in excess Make up to the mark with H_2O mix thoroly let stand for a few min , and filter thru a dry folded filter, discarding the first few cc of the filtrate To 100 cc of the filtrate in a 250 cc beaker add, with constant stirring, 10 cc of the tartaric acid soln 2 cc of glacial acetic acid and 23 cc of 95% (by volume) alcohol Cool in an ice bath, stir vigorously until the cream of tartar begins to crystallize and let stand in a refrigerator overnight Prepare a Gooch crucible, having a removable disk with a pad of asbestos about 10 mm thick Decant most of the liquid thru this filter wash the precipitate into the crucible with the KCl wash soln, and wash the beaker and precipitate 3 times using 20–30 cc of the wash soln in all Place the asbestos and precipitate in the beaker in which the precipitation was made and wash the crucible with about 50 cc of hot H_2O Heat the soln to boiling and titrate the hot soln with 0 1 *N* NaOH soln using phenolphthalein indicator Calculate the percentage of tartaric acid in the cheese by means of the formula—

$$\%\ \text{tartaric acid} = (14.26\ [0.015\ (B+1.5) - 1]\ n) \div g$$

in which

n = cc of tartaric acid in 10 cc of the tartaric acid soln reagent and

g = g of tartaric acid in 100 cc of the tartaric acid soln reagent and

B = cc of 0 1 *N* NaOH soln required for the titration

In the factor 14 26 the concentration caused by the insoluble solids of cheese of average composition is also taken into consideration

CITRIC ACID [1]

Qualitative Test—Tentative

103 To 10 g of the ground cheese add 20 cc of H_2O at a temp of about 50° and shake vigorously until the cheese is thoroly broken up Add 20 cc of H_2SO_4 (1+1) and shake vigorously Let stand for 5 min Add 20 cc of a 20% soln of phosphotungstic acid and shake vigorously Let stand for 5 min and filter To 20 cc of the filtrate add 10 cc of Br water and 5 cc of KBr soln and proceed with the oxidation as directed in the quantitative determination Add sufficient H_2SO_4 soln to dissolve the precipitated MnO If citric acid is present a heavy white precipitate that settles rapidly is formed

Quantitative Method—Tentative

REAGENTS

104 (a) *Sulfuric acid*.—Approximately 10% soln Dilute 6 cc of H_2SO_4 to 1 liter with H_2O

(b) *Sodium oxalate soln*.—Dissolve 2 g of Na-oxalate in 100 cc of H_2O

(c) *Finely powdered anhydrous sodium sulfite*

(d) *Phosphotungstic acid soln*.—Dissolve 20 g of phosphotungstic acid in H_2O and dilute to 100 cc

(e) *Potassium bromide soln*.—Dissolve 1 g of KBr in 40 cc of H_2O

(f) *Potassium permanganate soln*.—Dissolve 1 g of $KMnO_4$ in H_2O and dilute to 100 cc

filtrate become clear Return the cloudy filtrate to the filter and wash the receiving container twice with clear filtrate, returning the washings to the filter For whole eggs or for mixed whites and yolks, add 5 cc of 0 01 *N* acetic acid soln, mix gently, remove 1 drop of the egg soln to a spot plate containing 2 drops of H_2O, and add 1 drop of cresol red indicator Repeat the addition of 5 cc of acetic acid soln, mixing and testing with the indicator on the spot plate until the coloration disappears Fill to the mark with H_2O, shake gently, and filter thru an 18½ cm fluted filter paper, returning the filtrate to the filter if it is cloudy

8 DETERMINATION

Determine N in 50 cc of the filtrate as directed under II, 24, making certain that the free flame does not touch the flask above the level of the liquid Heat with a moderate flame for one hour after clearing Distil into 20 cc of 0 1 *N* acid Run a blank on the reagents

Pipet off 100 cc of the above filtrate into a 200 cc flask, add 15 cc of the NaCl soln, and fill almost to the mark with ethyl alcohol Mix, cool to room temp , make up to volume with alcohol, mix well, and allow to stand overnight Pipet off the supernatant liquid and filter thru an 18½ cm fluted filter paper Determine N in 100 cc of the filtrate as above To avoid bumping, add glass beads or delay the addition of K_2SO_4 and HgO until the alcohol has been boiled off Subtract the value obtained from the water-soluble N to obtain the water soluble N precipitable by 40% alcohol

FAT (ACID HYDROLYSIS METHOD)[5]—TENTATIVE

9 PREPARATION OF SOLUTION

Liquid eggs —Weigh accurately by difference approximately 5 g of the well mixed sample into a 50 cc beaker, add 10 cc of HCl and mix well Set the beaker in a water bath held at 75–80°, and stir at frequent intervals for 15–25 min , or until the sample is sufficiently hydrolized to form a clear soln Add 10 cc of 95% alcohol and cool

Powdered dried eggs —Weigh accurately 2 g of the well mixed sample into a 50 cc beaker, add 2 cc of 95% alcohol, and stir to moisten all particles (the moistening of the sample with alcohol prevents lumping on addition of the acid) Add 10 cc of HCl (25+13), mix well, set the beaker in a water bath held at 75–80°, and stir at frequent intervals for 15–25 min , or until the sample is sufficiently hydrolized to form a clear soln Add 10 cc of 95% alcohol and cool

10 DETERMINATION

Transfer the mixture to a Rohrig or Mojonnier fat extraction apparatus Rinse the beaker into the extraction tube with 25 cc of ethyl ether in 3 portions and shake the mixture well Add 25 cc of redistilled petroleum ether (b p below 60°) and mix well Let stand until the ether layer is practically clear Thru a filter consisting of a pledget of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether, draw off as much as possible of the ether fat soln into a weighed 125 cc beaker flask containing some porcelain chips Before weighing the beaker flask, dry it in an oven at the temp of boiling H_2O and then allow it to stand in the air to constant weight Re extract the liquid remaining in the tube twice, each time with only 15 cc of each ether Shake well on the addition of each ether Draw off the clear ether solns thru the filter into the same flask as before and wash the tip of the spigot, the funnel and the end of the funnel stem with a small quantity of a mixture of the two ethers in equal parts and free from suspended H_2O Evaporate the ethers slowly on a steam bath, then dry the fat in a boiling-water oven to constant weight (approximately 90 min) Remove the fat flask from the oven, allow it to stand in the

air until no further change in weight takes place, and weigh Correct this weight by a blank determination on the reagents used

11 LIPOIDS[a]—TENTATIVE

(a) *Liquid eggs* —Weigh accurately by difference approximately 10 g of the well-mixed sample into a 200 cc nursing bottle, add 100 cc of anhydrous ether, stopper with a softened cork, and shake vigorously Add five 5 cc portions of 95% alcohol and shake after each addition (The gradual addition of alcohol with shaking coagulates the proteins to a very fine state) Centrifugalize and decant the liquid into a 250 cc beaker containing some bits of broken porcelain Wash the neck of the bottle with ether, and place the beaker with the fat soln on the steam bath Add 15 cc of 70% alcohol to the egg residue in the bottle in a manner to wash down any particles adhering to the sides and set in a water bath held at 70–80° for 15 min Shake occasionally with a rotary motion so as to moisten all particles with the alcohol Cool and add 30 cc of ether, stopper, shake for 5 min , centrifugalize to throw down suspended particles, and decant the liquid into the original 250 cc beaker Rinse the bottle neck with ether Re extract the residue with 2 successive 20 cc portions of ether, shaking 1 min each time, centrifugalize, and decant into the beaker containing the first extract Evaporate the combined ether alcohol extracts just to dryness on the steam bath Drive off any remaining apparent moisture on the sides of the beaker by placing in a boiling water oven for about 5 min Dissolve the dried extract in about 15 cc of $CHCl_3$ and filter the soln into a previously dried and weighed flat bottomed Pt dish thru a pledget of cotton packed in the stem of a funnel Free any solid extract adhering to the beaker with a glass rod and transfer thru the filter into the Pt dish by means of $CHCl_3$ from a wash bottle all soluble extract from the beaker bottom and sides Finally wash the funnel and stem tip (The filtrate should be perfectly clear) Evaporate the $CHCl_3$ on the steam bath (an electric fan may be used to hasten evaporation) and dry the dish and contents in a boiling water oven to constant weight (approximately 90 min) Weigh and report the extract as lipoids

(b) *Powdered dried egg* —Transfer about 2 g of well mixed sample, accurately weighed, to a funnel having a pledget of cotton loosely placed in the stem Wash with ether 4 or 5 times to extract most of the ether soluble substances Collect the washings in a 250 cc beaker containing some bits of broken porcelain and place on the steam bath Transfer the residue and cotton in the funnel to a small glass mortar and allow the ether to evaporate at room temp Add 2–3 g of precipitated $CaCO_3$ of the egg residue, grind to a fine powder, and transfer the mixture to a 200 cc nursing bottle Wash the mortar pestle, funnel, and funnel stem tip with ether and add the washings to the original ether extract Continue as directed under (a), beginning with "Add 15 cc of 70% alcohol to the egg residue in the bottle

12 LIPOID PHOSPHORIC ACID (P_2O_5)—TENTATIVE

Dissolve the lipoids in 10–15 cc of $CHCl_3$ add 10–20 cc of 1% alcoholic KOH soln evaporate to dryness on a steam bath, and char completely in a furnace at a faint red heat Cover the dish with a cover glass, add sufficient HNO_3 (1+3) to make the soln slightly acid, and filter into a 100 cc volumetric flask Wash the filter and residue carefully, make up the filtrate to 100 cc and determine the P_2O_5 as directed under H, 7 or 10 For the volumetric method pipet 20 cc of the soln into a 250 cc beaker neutralize with NH_4OH (1+3) and then slightly acidify with HNO_3 (1+3) Set the beaker in a water bath held at 15–50 and add 15 g of NH_4NO_3 When the soln has reached the temp of the bath, add sufficient NH_4-molybdate soln previously heated to 45–50°, to precipitate all the phosphates stir, and heat for 30 min Filter the precipitate on an asbestos mat in a Hirsch funnel, wash with cold H_2O

and proceed as directed under II, 10 (a) beginning with "Transfer the precipitate and filter to the beaker or precipitating vessel" Report as lipoid P_2O_5

13 UNSAPONIFIABLE MATTER[8]—TENTATIVE

Extract the lipoids according to the directions given in 12 Determine the unsaponifiable matter in the extracted lipoids by the official F A C method (XXXI, 37) [8]

METHODS FOR DETECTION OF DECOMPOSITION

ACIDITY OF FAT[9]—OFFICIAL

(Not applicable to egg white)

14 REAGENTS

(a) *Anhydrous ether* —Prepare in the usual way from ordinary ethyl ether

(b) *Benzene* —Use the best available quality of benzene If it is not neutral, titrate 50 cc with the 0 05 *N* Na ethylate and correct subsequent results accordingly

(c) 0 05 *N sodium ethylate* —Dissolve a piece of metallic Na, approximately 1 cc in volume, in 800 cc of absolute alcohol Titrate 10 cc of 0 1 *N* HCl with this soln and add the calculated volume of absolute alcohol to make the soln 0 05 *N* Ascertain the normality factor by titration against 0 1 *N* HCl on the day the soln is used

15 DETERMINATION

(a) *Dried eggs* —Weigh in a tared Al dish about 63 mm (2½ inches) in diameter 2 g of the powdered sample and dry at 55 under a pressure not exceeding 125 mm (5 inches) of Hg Weigh to the third decimal place at the end of 2 hours and make further weighings at ½-hour intervals until no further loss in weight occurs Extract the dried residue with anhydrous ether, preferably in a Knorr apparatus Carefully transfer the egg powder to a 12 5 cm hardened filter paper, fold the paper once, place it on a 15 cm qualitative filter paper, and roll the papers and contents into a cylinder that will fit snugly into the extraction tube folding in one end of the cylinder to prevent loss of material (An asbestos plug is not needed in the extraction tube, and if the extractor is working rapidly, 3 hours is sufficient to insure proper extraction) Evaporate the ether from the extraction flask, dry the extract for 1 hour at 55° under a pressure not exceeding 125 mm, and weigh to the third decimal place Dissolve the extract in 50 cc of benzene, add 3 to 4 drops of phenolphthalein indicator, and titrate with the standard Na ethylate soln The end point is reached when the yellow color changes to orange Express the result as the number of cc of 0 05 *N* Na ethylate required per g of ether extract

(b) *Liquid eggs* —Weigh to the third decimal place in a weighed lead dish about 5 g of the sample and dry as directed under (a) Weigh after drying for about 5 hours and thereafter, at 1 hour intervals, until no further loss in weight occurs To prepare the dried residue for extraction with ether place the lead dish upon a 12 5 cm hardened filter paper cut the sides of the dish thru at 4 equidistant points, and flatten down Place another similar filter paper on top of the lead dish and its contents and roll the papers and dish into a cylinder that will fit snugly into the extractor, folding in one end of the cylinder to prevent any of the egg residue from dropping into the extraction flask Proceed thereafter as directed under dried eggs

PHOSPHORIC PENTOXIDE[10]—TENTATIVE

16 REAGENTS

(a) *Sodium carbonate soln* —Dissolve 10 g of Na_2CO_3 in H_2O and dilute to 100 cc

(b) *Olive oil*

PREPARATION OF SOLUTION

17 From the well mixed sample, weigh accurately, by difference, approximately 2 g of yolk, 4 g of whole eggs, or 10 g of whites into a 250 cc low form Pyrex beaker Add 20 cc of the Na_2CO_3 soln and evaporate to dryness on an electric hot plate or overnight at 100–105° Add to the whites 0 5 cc of olive oil Transfer the beaker while hot to an electric muffle heated to 500 (faint redness), and allow it to remain at this temp for 1 hour Cool, add a few drops of H_2O break up the charge with a glass rod with a flattened end and cover the beaker with a watch glass Then add slowly and with continuous stirring 10 cc of HNO_3 (1+3) and filter, collecting the filtrate in a 300 cc or 500 cc Erlenmeyer flask Thoroly wash the charred material and filter with H O from a wash bottle

DETERMINATION

18 In the prepared filtrate determine the P_2O_5 as directed under 12 and 11, 10 (a), using 10–50 cc of the molybdate soln Report as total P_2O_5

CHLORINE—TENTATIVE

REAGENTS

19 (a) *Sodium carbonate soln* —Dissolve 10 g of Na_2CO_3 in H_2O and dilute to 100 cc

(b) *Olive oil*

PREPARATION OF SOLUTION

Liquid Eggs —From the well mixed sample weigh accurately, by difference, ap proximately 4 g of yolk or 7 g of whole eggs or 10 g of whites into a 150 cc low form Pyrex beaker, add 20 cc of the Na_2CO_3 soln mix, and evaporate to dryness on an electric hot plate or overnight at 100 Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow it to remain at that temp for 1 hour Cool add a few drops of H_2O, and break up the charge with a glass rod Add 50 cc of H_2O cover the beaker with a watch glass, add slowly 20 cc of HNO_3 (1+3), mix remove the watch glass and filter, collecting the filtrate in a 200 cc volumetric flask Wash the charred material and filter thoroly with H_2O from a wash bottle keeping the total volume of filtrate to 180 cc or less

Dried Eggs —From the well mixed sample, transfer 2 g of whole eggs or yolks or 1 g of whites into a 150 cc low form Pyrex beaker and proceed as directed under *Liquid Eggs*

DETERMINATION

Add 10 cc of 0 1 *N* silver nitrate soln to the prepared filtrate and proceed as di rected under XII, 35

22 EXTRACTION AND IDENTIFICATION OF ADDED COLOR

Determine as directed under XX, 72

23 DETECTION OF THE PRESENCE OF WHOLE EGG OR COMMERCIAL EGG YOLK SOLIDS

Determine as directed under XX, 73

24 ESTIMATION OF THE PERCENTAGE OF EGG SOLIDS

Determine as directed under XX 74

SELECTED REFERENCES

[1] J Assoc Official Agr Chem , 6, 5 (1922), 8, 599 (1925), 9, 56 (1926)
[2] Ibid , 6, 6 (1922), 8, 600 (1925), 9, 56, 354 (1926), U S Dept Agr Bull , 846, p 89
[3] J Assoc Official Agr Chem , 8, 601 (1925), 9, 57 (1926)
[4] Ibid , 7, 85 (1924), 8, 620 (1925), 12, 56 (1929), 14 (1931)
[5] Ibid , 8, 601 (1925), 9, 58 (1926)
[6] Ibid , 7, 91 (1923), 8, 602 (1925), 9, 58 (1926)
[7] Ibid , 7, 91 (1923), 8, 603 (1925), 9, 59 (1926)
[8] Ibid , 9, 45, 124 (1926)
[9] Ibid , 6, 6 (1922), 10, 50 (1927)
[10] Ibid , 14 (1931)

XXIV FISH AND OTHER MARINE PRODUCTS*

* See note at bottom of p xv

XXV FLAVORING EXTRACTS

VANILLA EXTRACT AND ITS SUBSTITUTES

1 SPECIFIC GRAVITY*—OFFICIAL

Determine the specific gravity at 20/4° by means of a pycnometer, directed as under XVII, 24

2 ALCOHOL—OFFICIAL

Proceed as directed in 10 and under XVII, 59, 60 and 61

3 GLYCEROL—TENTATIVE

Proceed as directed under XVII, 26, 27 and 28, the method selected depending upon the quantity of sugar present using such a quantity of the sample as contains 0.1–0.4 g of glycerol

VANILLIN AND COUMARIN (GRAVIMETRIC) OFFICIAL

(This method is not applicable to concentrated vanillin and coumarin preparations in which the quantity of vanillin and coumarin present in 50 cc exceeds the quantity dissolved by 100 cc of H_2O at 20° Then use a smaller quantity of the sample and dilute to 50 cc)

4 PREPARATION OF SOLUTION

Measure 50 cc of the extract at 20° into a 250 cc beaker bearing marks showing volumes of 80 cc and 50 cc, dilute to 80 cc and evaporate to 50 cc in a water bath kept at 70° or below Dilute again with H_2O to 80 cc and evaporate to 50 cc Transfer to a 100 cc flask rinsing the beaker with hot H_2O add 2 cc of 5% neutral Pb acetate soln, make up to the mark with H_2O, shake, and allow to stand 15 hours (overnight) at 37–40 Decant into a small dry filter reserving the filtrate (Soln A) for the determination of vanillin and coumarin (5) the Ph number (8 and 9) and the residual color (20)

5 DETERMINATION

(a) Vanillin —Transfer a 40 cc aliquot of the filtrate (Soln A) to a separatory funnel and extract with 4 successive 15 cc portions of ether (previously washed twice with an equal volume of H_2O to remove alcohol) Wash the combined ether solns 4 or 5 times with NH_4OH (1+11) using 10 cc the first time and 5 cc thereafter Reserve the ether soln for the determination of coumarin Slightly acidify the combined ammoniacal solns with HCl (1+2) cool and extract in a separatory funnel with 4 portions of washed ether using about 40 cc altogether Evaporate the ethereal solns at room temp dry over H_2SO_4, and weigh (The vanillin residue often appears first in the form of oil like droplets, which on standing crystallize out light colored masses) If, after standing in a desiccator the residue is semi-liquid dissolves or forms gummy extract the vanillin from it by treating with at least 15 successive portions (each boiling petroleum ether (b p 40° or below) combine the petroleum ether extracts evaporate to dryness and weigh

The residue if pure vanillin should be white crystals melting at approximately 80° Dissolve a small quantity of the residue in 2 drops of HCl (sp gr 1 and 1 crystal of resorcin Vanillin gives a pink color ation

(b) Coumarin —Evaporate at room temp the ether extract obtained under (a) from which the vanillin has been removed by treatment with NH_4OH (1+11) dry over H_2SO_4 and weigh

* See p 1

The residue, if pure coumarin, melts at approximately 67° Dissolve a small quantity of the residue in not more than 0 5 cc of hot H_2O and add a few drops of 0 1 *N* I soln Coumarin yields a brown precipitate which finally gathers in green flecks leaving a clear brown colored soln The reaction is especially marked if the reagent is applied with a glass rod to a few drops of the soln on a white plate or tile

VANILLIN (COLORIMETRIC)[2]—OFFICIAL

6 REAGENTS

(a) *Standard vanillin soln* —Dissolve 0 1 g of pure vanillin in H_2O and dilute to 1 liter

(b) *Phosphotungstic-phosphomolybdic acid* —To 100 g of pure Na tungstate and 20 g of phosphomolybdic acid (free from nitrates and NH_4 salts), add 100 g of sirupy H_3PO_4 (containing 85% H_3PO_4) and 700 cc of H_2O Boil over a free flame for 1½–2 hours, cool, filter, if necessary, and make up with H_2O to 1 liter An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid

(c) *Sodium carbonate soln* —Prepare 1 liter of a saturated soln of pure Na_2CO_3

(d) *Lead soln* —Dissolve 50 g each of basic and neutral Pb acetate in H O and make up to 1 liter

7 DETERMINATION

Transfer to a 100 cc volumetric flask a quantity of the sample that contains from 8–12 mg of vanillin (usually 5 cc) Add 75 cc of tap H_2O at room temp and 4 cc of the Pb soln Dilute to 100 cc with H_2O and mix Filter thru a dry filter paper and pipet 5 cc of the clear filtrate into a 50 cc volumetric flask Into another 50 cc volumetric flask pipet 5 cc of the standard vanillin soln To each of these flasks add from a pipet 5 cc of Reagent (b), allowing the reagent to flow down the neck of the flask in such a way as to wash down the vanillin soln that may be on the sides of the flask Mix the contents of the flasks by rotating and after 5 min dilute contents to 50 cc with the Na_2CO_3 soln Mix thoroly by inverting the flasks several times and allow to stand for at least 10 min so that the precipitate that forms may separate completely Filter the solns thru dry filter papers and compare the blue colors of the clear solns in a colorimeter Report result as g of vanillin per 100 cc of extract

LEAD NUMBER

8 *Method I Winton*[3]*—Official*

To a 10 cc aliquot of the filtrate from the Pb acetate precipitate obtained (Sol in A, 4) add 15 cc of H_2O, 10 cc of H_2SO_4 (1+9) and 100 cc of 95% alcohol and stir Let stand overnight filter thru a weighed Gooch crucible, wash the precipitate with 95% alcohol, dry at a moderate heat, ignite at low redness for 3 min , taking care to avoid the reducing flame and weigh Conduct a blank determination, using H_2O containing 4 or 5 drops of glacial acetic acid in place of the sample Calculate the Pb number by the following formula and report as "Lead number—Winton"

$$P = \frac{100 \times 0\ 6832\ (S - W)}{5} = 13\ 66\ (S - W), \text{ in which}$$

P = Pb number (g of metallic Pb in the precipitate obtained from 100 cc of the sample),

S = g of $PbSO_4$ corresponding to 2 5 cc of the Pb acetate soln as determined in a blank analysis, and

W = g of $PbSO_4$ obtained in 10 cc of the filtrate from the Pb acetate precipitate as obtained in 4

Method II Wichmann[6]—Official

9 REAGENT

Lead acetate soln—Dissolve 80 g of neutral Pb acetate in H_2O that has been recently boiled dilute to 1 liter, and filter if the soln is not clear

10 DETERMINATION

Place 175 cc of boiled H_2O in a round bottomed flask of 1 liter capacity Add by means of pipets 25 cc of the Pb acetate soln and 50 cc of the sample Place the flask in a hole in an asbestos board that is large enough to prevent the heating of the upper portion of the flask (When the contents of the flask are reduced to 50 cc of liquid, the level of the liquid will be even with the top of the board, or slightly above it) Connect the flask to a condenser, and with a moderate flame distil 200 cc into a volumetric flask Calculate the approximate alcohol content of the extract from the specific gravity of the distillate (For accurate results, redistil over alkali) Transfer the residual soln to a 100 cc volumetric flask by means of CO_2 free H_2O and a bent glass rod provided with a rubber tip When cool, dilute to 100 cc with CO_2-free H_2O, mix, and filter thru a dry filter (Soln A)

Pipet 10 cc of Soln A into a 250 cc beaker, add 25 cc of H_2O, 2 cc of H_2SO_4 (1+1) and 100 cc of 95% alcohol, stir and allow to settle overnight Filter on a Gooch crucible, wash with 95% alcohol dry ignite at low redness cool in a desiccator, and weigh Conduct a blank determination using 5 drops of glacial acetic acid in place of the sample and distil 150 cc instead of 200 cc The difference between the two weights of $PbSO_4$ multiplied by 13 66 gives the Pb number of the extract Report as "Lead number—Wichmann"

Lead as Lead Chromate[6]—Official, first action

11 REAGENT

Potassium dichromate soln—Approximately 0 1 *N* Dissolve 5 g of pure crystallized $K_2Cr_2O_7$ in H_2O and dilute to 1 liter

DETERMINATION

Pipet 10 cc of the clear filtrate from the Pb precipitate (soln A, 4 or 10) to a 400 cc beaker and add 2 cc of glacial acetic acid, 25 cc of H_2O, and 25 cc of the $K_2Cr_2O_7$ soln Heat the beaker and contents immediately with a moderate flame and continue heating until the precipitate changes in color from yellow to orange Then filter the soln thru a weighed Gooch crucible provided with an asbestos mat and wash thoroly with hot H_2O and then with a few cc each of alcohol and ether Dry at 100 cool in a desiccator and weigh Determine the Pb in the blank in the same manner The difference in weights of $PbCrO_4$ multiplied by 12 52 is the Pb number

12 TOTAL SOLIDS—OFFICIAL

Proceed as directed under XXXIV, 4, using 10 cc of the sample

13 ASH—OFFICIAL

Evaporate 10 cc of the extract and determine the ash as directed under XXVII, 8

14 ASH CONSTITUENTS

Proceed as directed under XII

15 SUCROSE—OFFICIAL

Proceed as directed under XXXIV, 22, 23, or 28

VANILLA RESINS

16 *Quantitative Test[6]—Tentative*

Pipet 50 cc of the extract into a small beaker, add 50 cc of H_2O, and evaporate to 50 cc on the steam bath Add 50 cc of H_2O and again evaporate to 50 cc Cool If the mixture has an acid reaction, add 2 cc of HCl (1+1) If the mixture is not acid to litmus, add HCl (1+1), dropwise, until distinctly acid to litmus paper, then 1 cc in excess Cover and let stand overnight Filter, wash 6 or 7 times with approximately 0 05 *N* HCl [9 cc of HCl (1+1) per liter of H_2O] Dissolve the resin in warm 95% alcohol by pouring thru the filter Evaporate the alcohol in a tared 50 cc beaker and dry to constant weight at 100° Report results to 2 decimal places only Reserve the resin for qualitative tests

17 QUALITATIVE TEST—TENTATIVE

Place a portion of the dried residue in a few cc of 5% KOH soln Vanilla resins dissolve giving a deep red soln Acidify, and a precipitate is obtained

Dissolve a portion of the dried residue in 95% alcohol To a portion of the soln add a few drops of a 10% $FeCl_3$ soln, to another portion add HCl Neither produces any marked change in color if the residue consists of vanilla resins Most other resins in alcoholic soln give color reactions with $FeCl_3$ or HCl

To a portion of the filtrate obtained (16), add a few drops of basic Pb acetate soln [XXXIV, 18 (a)] Owing to the excessive quantity of organic acids, gums, and other extractive matter, the precipitate is so bulky as almost to solidify The filtrate from this precipitate should be almost colorless

Test another portion of the filtrate from the resin for tannin with a soln of gelatin Tannin is present in varying but small quantities, but should not be present in excessive quantities

18 METHYL ALCOHOL—OFFICIAL

Proceed as directed under XVII, 73, 74 or 75, using the distillate from the determination of alcohol under 2

19 COLOR VALUE—TENTATIVE

Pipet 2 cc of the extract into a 50 cc volumetric flask and dilute to the mark with a mixture of equal parts of 95% alcohol and H_2O Determine the color value of this diluted extract in terms of red and yellow by means of a Lovibond tintometer, using a 1-inch cell To obtain the color value of the original extract multiply the figures for each color by 25

20 RESIDUAL COLOR AFTER PRECIPITATION WITH LEAD ACETATE[7]—TENTATIVE

Determine the color value in terms of red and yellow, of the filtrate from the Pb acetate precipitate obtained under 4, using a 1-inch Lovibond cell Multiply the reading by 2 to reduce the results to the basis of the original extract If the actual reading of the soln is greater than 5 red and 15 yellow, as may happen if the extract is highly colored with caramel, a half or quarter inch cell should be used, and the readings multiplied, respectively, by 4 or 8 To obtain the percentages of the two colors remaining in the Pb-acetate filtrate, divide the figures for red and yellow, respectively, by the corresponding figures of the original extract obtained under 19 and multiply the quotients by 100 Calculate also the ratio of red to yellow in both extract and Pb acetate filtrate

21 COLORS INSOLUBLE IN AMYL ALCOHOL—TENTATIVE

Proceed as directed under XVII, 79, using 25 cc of the extract and shaking with 25 cc of the Marsh reagent instead of 20 cc

22 COLORING MATTERS OTHER THAN CARAMEL—TENTATIVE

Proceed as directed under XXI

LEMON AND ORANGE EXTRACTS

SPECIFIC GRAVITY*—OFFICIAL

23 Determine the specific gravity at 20/4° by means of a pycnometer, as directed under XVII, 24

ALCOHOL

Method I—Official

24 Pipet 50 cc of the extract, measured at 20°, into a 200 cc volumetric flask dilute with H_2O to approximately 200 cc, and allow the mixture to stand until the oil separates in a clear layer at the top, or centrifugalize and add H_2O to bring the lower meniscus of the oil to the mark Pour the mixture into a dry Erlenmeyer flask containing 5 g of light $MgCO_3$, stopper, shake well, and filter quickly thru a large dry, folded filter Introduce a 150 cc aliquot of the filtrate, measured at 20°, into a 300–500 cc distillation flask, attach the flask to a condenser, and distil almost 100 cc Add H_2O to complete the volume of the distillate to 100 cc at 20°, mix well, and determine the specific gravity at 20/1° Ascertain the corresponding percentage of alcohol by volume from XLII, Tables 19-21, and multiply the result thus obtained by 2½ to obtain the percentage of alcohol by volume in the original sample

Method II[a]*—Official*

25 (Applicable to extracts consisting only of oil, alcohol, and water)

Let S represent the specific gravity of the extract at 20/4°, as determined under 23, O the specific gravity of the oil and P, the percentage of oil found Then $100 - P$ will be the percentage of the water alcohol soln, the specific gravity of which represented by I, is calculated as follows

$$S = \frac{OP + I(100 - P)}{100} \quad \text{whence} \quad P = \frac{100S - OI}{100 - I}$$

The value of I, the alcohol equivalent of P, is obtained from XLII, Tables 19 21 It gives the percentage of alcohol in the alcohol water soln To find the percentage of alcohol in the extract, apply the following formula

$$\text{Percentage by volume of alcohol in the extract} = E\left(1 - \frac{P}{100}\right)$$

The value of O for lemon oil may be taken as 0 86 and for orange oil as 0 8

GLYCEROL—TENTATIVE

26 I roceed as directed under XVII, 26, 27 or 28, the method selected depending upon the quantity of sugar present Use a quantity of the sample that contains 0 1–0 4 g of glycerol

OILS OF LEMON AND ORANGE IN EXTRACTS

I Polarization—Official

27 Without diluting polarize the extract at 20 in a 200 mm tube Divide the reading in degrees Ventzke by 3 – in the case of lemon extract and by 5 2 in the case of orange extract in the absence of other optically active substances the result will be the percentage of oil by volume If cane sugar is present determine as directed under 35 and correct the reading accordingly To obtain the percentage of oil by weight from the percentage by volume multiply the volume percentage by 0 86 in

[a] See p 4 of 9 p s

the case of lemon extracts, and by 0 85 in the case of orange extracts, and divide the results by the specific gravity of the original extract

28 *II By Precipitation—Official*

Pipet 20 cc of the extract into a Babcock milk bottle Add 1 cc of HCl (1+1), then 25–28 cc of H_2O previously warmed to 60°, mix, and let stand in H_2O at 60° for 5 min Centrifugalize for 5 min , fill the bottle with warm H_2O to bring the oil into the graduated neck of the flask, again centrifugalize for 2 min , and place the flask in H_2O at 60° for a few min Note the percentage of oil by volume If oil is present in amounts over 2%, add 0 4% to the percentage of oil noted to correct for the solubility of the oil If less than 2% and more than 1% is present, add 0 3% for this correction To obtain the percentage of oil by weight from the percentage by volume multiply the volume percentage by 0 86 in the case of lemon extracts, and by 0 85 in the case of orange extracts, and divide the result by the specific gravity of the original extract

TOTAL ALDEHYDES[1]—OFFICIAL

29 REAGENTS

(a) *Aldehyde free alcohol* —Allow 95% alcohol, containing 5 g of metaphenylenediamine hydrochloride per liter, to stand for at least 24 hours with frequent shaking (Nothing is gained by previous treatment with KOH) Boil under a reflux condenser for at least 8 hours, longer if necessary, allow to stand overnight, and distil rejecting the first 10 and the last 5 cc which come over Store in a dark, cool place in well filled bottles Twenty-five cc of this alcohol, on standing 20 min at 14–16° with 20 cc of the sulfite fuchsin soln should develop only a faint pink coloration If a stronger color is developed, repeat the treatment with metaphenylenediamine hydrochloride as above

(b) *Sulfite fuchsin soln* —Dissolve 0 5 g of fuchsin in 250 cc of H_2O, add an aqueous soln of SO_2 containing 16 g of the gas, allow to stand until colorless or nearly so, and make up to 1 liter with H_2O Let stand 12 hours before using and keep in a refrigerator This soln is liable to deteriorate and should be reasonably fresh when used

(c) *Standard citral soln* —Weigh 0 5 g of citral into a 50 cc volumetric flask, make up to mark with the aldehyde free alcohol at room temp , stopper the flask, and mix by shaking Dilute 10 cc of this soln with the aldehyde free alcohol to 100 cc in a volumetric flask, stopper the flask and mix by shaking 1 cc of the dilute soln = 1 mg of citral

30 DETERMINATION

Weigh approximately 25 g of the extract in a stoppered weighing flask, transfer to a 50 cc volumetric flask, and dilute to the mark at room temp with aldehyde free alcohol Measure, at room temp , 2 cc (or other suitable quantity) of this soln into a comparison tube Add 25 cc of the aldehyde free alcohol (previously cooled to 14–16°), then 20 cc of the sulfite fuchsin soln (also cooled) and finally make up to the 50 cc mark with aldehyde free alcohol Mix thoroly, stopper, and keep at 14–16 for 15 min Prepare a standard for comparison at the same time and in the same manner, using 2 cc of the standard citral soln, and compare the colors developed Calculate the amount of citral present and repeat the determination using a quantity sufficient to give the sample approximately the strength of the standard From this result calculate the quantity of citral in the sample If the comparisons are made in Nessler tubes, standards containing 1, 1 5, 2, 2 5, 3, 3 5 and 4 mg of citral may be prepared and the trial comparison made against these, the final comparison being made with standards lying between 1 5 and 2 5 mg with 0 25 mg increments

FLAVORING EXTRACTS

It is absolutely essential to keep the reagents and comparison tubes at the re quired temp , 14–16 If the comparisons are made in a bath (this being possible only when the bath is of glass) the standards should be discarded within 20 min after adding the sulfite fuchsin soln Give samples and standards identical treatment

CITRAL[a]—OFFICIAL FIRST ACTION

(Lemon and orange extracts)

REAGENTS

31

(a) *Metaphenylenediamine hydrochloride oxalic acid soln* —Dissolve 1 g of meta phenylenediamine hydrochloride in about 45 cc of 85% alcohol and 1 g of crystal lized oxalic acid in a similar quantity of alcohol of the same strength and pour the two solns into a 100 cc volumetric flask Add 2 or 3 g of fullers earth, dilute to the mark with 85% alcohol mix, and filter thru a double folded filter

(b) *Alcohol* —95% by volume for terpeneless lemon and orange extracts

(c) *Standard citral soln* —Dissolve 1 g of citral in alcohol, 90–95% by volume, dilute to 100 cc and mix Dilute 5 cc of this soln to 50 cc with alcohol, 90–95% by volume and mix 1 cc of the second soln = 1 mg of citral

DETERMINATION

32

Weigh 25 g of the extract into a 50 cc volumetric flask, dilute to the mark with al cohol and mix Pipet 2 cc or other suitable quantity of this soln into a colorimeter tube add 10 cc of Reagent (a), dilute to suitable volume and compare the resulting color with the colors of a set of standards containing known quantities of citral pre pared like the sample

TOTAL SOLIDS—OFFICIAL

33

Proceed as directed under XVII, 62, using 10 cc of the sample measured at 20°

ASH—OFFICIAL

34

Ignite the residue from 10 cc of the extract as directed under XXVII, 8

SUCROSE—OFFICIAL

35

Neutralize the normal weight of the extract evaporate to dryness wash several times with ether, dissolve in H_2O and determine as directed under XXXIV, 22, 23 or 28

METHYL ALCOHOL—OFFICIAL

36

Proceed as directed under XVII, 73, 74 or 75, using the distillate from the deter mination of alcohol under 24

COLORING MATTERS—TENTATIVE

37

Proceed as directed under XXI

LEMON AND ORANGE PEEL COLOR—TENTATIVE

38

Place a few cc of the extract in each of 2 test tubes to one, add slowly 3–4 vol umes of HCl and to the other, several drops of NH_4OH If the color is due to lemon or orange peel only, it is materially deepened by each treatment

LEMON AND ORANGE OILS

SPECIFIC GRAVITY*—OFFICIAL

39

Determine the specific gravity at 20/4° by means of a pycnometer as directed under XVII, 24

* See also p xvii

40 INDEX OF REFRACTION—OFFICIAL

Determine the index of refraction with any standard instrument, making the reading at 20° (cf XXXI, 8)

41 OPTICAL ROTATION—OFFICIAL

Determine the rotation at 20° with any standard instrument, using a 50 mm tube and Na light The results should be stated in angular degrees on a 100 mm basis If instruments having the sugar scale are used, the reading for orange oils is above the range of the scale, but readings may be obtained by the use of standard levorotatory quartz plates, or by the use of a 25 mm tube The true rotation cannot be obtained by diluting the oil with alcohol and correcting the rotation in proportion to the dilution

42 TOTAL ALDEHYDES[11]—OFFICIAL

Weigh a small quantity of the sample into a small stoppered flask and dilute with aldehyde free alcohol in the proportion of 2 g of lemon oil or 4 g of orange oil to 10 cc of soln Determine the total aldehydes as directed under 30, expressing the result as citral

Kleber Method[12]*—Official*

43 REAGENTS

(a) *Phenylhydrazine soln* —Prepare a 10% soln in absolute alcohol A sufficiently pure product can be obtained by distilling the commercial article in vacuo, rejecting the first portions coming over that contain NH_3

(b) *Hydrochloric acid* —0 5 *N*

44 DETERMINATION

Weigh accurately about 15 g of the sample into a small, glass stoppered flask, and add 10 cc of the phenylhydrazine soln Allow to stand 30 min at room temp and titrate with the 0 5 *N* HCl, using either methyl or ethyl orange indicator Titrate similarly 10 cc of the phenylhydrazine soln The difference in the number of cc of 0 5 *N* acid used in these 2 titrations, multiplied by the factor 0 076, gives the weight of citral in the sample If difficulty is experienced in detecting the end point of the reaction, titrate until the soln is distinctly acid, transfer to a separatory funnel, and draw off the alcoholic portion Wash the oil with H_2O, adding the washings to the alcoholic soln, titrate back with 0 5 *N* alkali, and make the necessary corrections

45 *Hiltner Method*[12]*—Official*

Weigh accurately about 2 g of lemon oil or 8 g of orange oil into a 100 cc volumetric flask, dilute to the mark with 95% alcohol, and proceed as directed under 32, using 2 cc of the dilute soln for the comparison

46 PHYSICAL CONSTANTS OF THE 10 PER CENT DISTILLATE[13]—OFFICIAL

Place 50 cc of the sample in a 3 bulb Ladenburg flask having the main bulb 6 cm in diameter and of 120 cc capacity and the condensing bulbs of the following dimensions 3 5 cm, 3 cm, and 2 5 cm The distance from the bottom of the flask to the opening of the side arm should be 20 cm Distil the oil at the rate of 2 cc per min until 5 cc has been distilled Determine the refractive index and rotation of this distillate as directed under 40 and 41

47 PINENE[14]—OFFICIAL

Mix the 10% distillate obtained under 46 with 5 cc of glacial acetic acid, cool the mixture thoroly in a freezing bath, and add 10 cc of ethyl nitrite Then add slowly,

with constant stirring, 2 cc of HCl (2+1) Keep the mixture in the freezing bath 15 min Collect the crystals formed on a filter, using suction, and wash with 95% alcohol Return the combined filtrate and washings to the freezing bath for 15 min Collect the additional crystals formed on the original filter Wash the combined crops of crystals thoroly with alcohol Dry at room temp and dissolve in a minimum quantity of $CHCl_3$ Add methyl alcohol to the $CHCl_3$ soln, a little at a time until the nitroso chlorides crystallize out Mount the separated and dried crystals in olive oil and examine under the microscope Pinene nitroso chloride crystals have irregular pyramidal ends, while limonene nitroso chloride crystallizes in needles

ALMOND EXTRACT

ALCOHOL—TENTATIVE

48 As almond extract usually contains only about 1% of almond oil, in most cases the alcohol can be calculated from the specific gravity of the extract If the extract is high in solids, determine the alcohol as follows Add 25 cc of the extract, measured at 20°, to 75 cc of saturated NaCl soln in a separatory funnel and extract twice with 50 cc portions of petroleum ether (b p 40–60) Collect the petroleum ether extract in a second separatory funnel, and wash twice with 2 portions (25 cc) of saturated brine Combine the original salt soln with the washings, add a little powdered pumice and distil into a 100 cc volumetric flask When almost 100 cc has been distilled make up to the mark with H_2O at 20° and determine alcohol from the specific gravity, as directed under XVII, 61

BENZALDEHYDE—TENTATIVE

REAGENT

49 *Phenylhydrazine soln*.—Add 3 cc of glacial acetic acid to 40 cc of H_2O and mix with 2 cc of phenylhydrazine

DETERMINATION

50 Measure out 2 portions of 10 cc each of the extract into 300 cc Erlenmeyer flasks and add 10 cc of the phenylhydrazine soln to one flask and 15 cc to the other Allow the mixtures to stand overnight in a dark place Then add 200 cc of H_2O, and filter thru a weighed Gooch crucible provided with a thin layer of asbestos Wash the precipitate first with cold H_2O and finally with 10 cc of 10% alcohol Dry at 70° for 3 hours at a pressure not to exceed 100 mm of Hg or to constant weight over H_2SO_4 The weight of the precipitate multiplied by the factor 5 408, gives the weight of benzaldehyde in 100 cc of the sample If duplicate determinations do not agree, repeat the operation, using a larger quantity of the phenylhydrazine soln

BENZOIC ACID[a]—TENTATIVE

51 Measure 10 cc of the extract into a 100 cc flask and add 10 cc of a 10% NaOH soln and 20 cc of 3% H_2O_2 soln, cover with a watch glass and place in a water oven Oxidation of the aldehyde to benzoic acid begins almost immediately and should be continued 9–10 min after all odor of benzaldehyde has disappeared (usually 20–30 min) Remove the flask from the water oven, transfer the contents to a separatory funnel, rinsing off the watch glass, add 10 cc of H_2SO_4 (1+5), and cool the contents of the funnel to room temp under the water tap Extract the benzoic acid with 4 portions of 25 20 20 and 20 cc of ether, respectively and wash the combined extracts with 2 portions of 9–10 cc of H_2O or until all H_2SO_4 is removed Filter into a weighed dish evaporate at room temp , dry overnight in a desiccator and weigh the benzoic acid Multiply the result by 10

Multiply the grams per 100 cc of benzaldehyde obtained under 50 by 1 151 to obtain the equivalent of benzoic acid and subtract this product from the grams per 100 cc of total benzoic acid obtained above The difference is the grams of benzoic acid per 100 cc of the extract

HYDROCYANIC ACID

52 *Qualitative Test—Tentative*

Add several drops of a freshly prepared 3% $FeSO_4$ soln and a single drop of 1% $FeCl_3$ soln to several cc of the extract Mix thoroly and add 10% NaOH soln, dropwise until no further precipitate forms and then H_2SO_4 (1+9) to dissolve the precipitate In the presence of even small quantities of HCN, a Prussian blue coloration or suspension will develop

53 *Quantitative Method—Tentative*

(In the absence of chlorides)

Measure 25 cc of the extract into a small flask and add 5 cc of freshly precipitated $Mg(OH)_2$ (Cl free) Titrate with 0 1 *N* $AgNO_3$ soln, using K_2CrO_4 as an indicator 1 cc of 0 1 *N* $AgNO_3$ = 0 0027 g of HCN

NITROBENZOL

54 *Qualitative Test—Tentative*

Boil a few cc of the extract with some Zn dust and acetic acid and filter Add to the filtrate a drop of $CHCl_3$ make strongly alkaline with 10% NaOH soln, and heat The presence of nitrobenzol in the original extract is indicated by the development of the characteristic odor of phenylisonitrile

CASSIA, CINNAMON, AND CLOVE EXTRACTS

55 ALCOHOL—TENTATIVE

Determine as directed under 48

56 OIL[15]—TENTATIVE

Transfer 10 cc of the extract to a separatory funnel, add 30 cc of H_2O, acidify with 1 cc of HCl (1+1), and extract 3 times with ether, using not less than 100 cc altogether Wash the combined ether solns twice with H_2O, and in the case of cinnamon extract dry by shaking with a small quantity of granulated CaCl Transfer to a weighed wide mouthed weighing bottle and evaporate the ether as rapidly as possible on a boiling water bath, rotating the liquid upon the sides of the bottle in order to rid the residual oil of traces of ether Weigh the residue and divide the weight by the specific gravity of the oil in order to obtain the percentage of oil by volume In the case of clove oil, allow the weighing bottle to remain in the balance case until the usual film of moisture has evaporated The time of weighing however should not be delayed over 3 min Determine the refractive index of the residual oils at 20 Dissolve a drop of the oil in several drops of alcohol and add a drop of 10% $FeCl_3$ soln

The following tabulation gives the specific gravity refractive index at 20 and color reaction with $FeCl_3$ soln

OIL	SPECIFIC GRAVITY	REFRACTIVE INDEX AT 20°	COLOR REACTION WITH FERRIC CHLORIDE SOLUTION
Cassia	1 05	1 585–1 600	Brown
Cinnamon	1 03	1 590–1 599	Green
Cloves	1 055	1 560–1 565	Deep blue

the case of wintergreen, use as a floating medium a mixture of 1 volume of concentrated H_2SO_4 and 3 of saturated Na_2SO_4 soln

63 METHYL SALICYLATE IN WINTERGREEN EXTRACT[16]—TENTATIVE

Mix 10 cc of the extract with 10 cc of 10% KOH soln Heat on a steam bath until the volume is reduced about one half Add a distinct excess of dilute HCl (1+1), cool, and extract with 3 portions of ether, 40, 30, and 20 cc, respectively Filter the extract thru a dry filter into a weighed dish, wash the paper with 10 cc of ether, and allow the filtrate and washings to evaporate spontaneously Dry in a desiccator containing H_2SO_4 and weigh Multiply the weight of salicylic acid so found by 9 33 to obtain the percentage by volume of methyl salicylate in the sample

ANISE AND NUTMEG EXTRACTS

64 OIL[16]—TENTATIVE

To 10 cc of the extract in a Babcock milk bottle, add 1 cc of HCl (1+1), then sufficient half saturated salt soln, previously heated to 60°, to fill the flask nearly to the neck Cork and let stand in H_2O at 60° for about 15 min , rotate occasionally, and centrifugalize for 10 min at about 800 r p m Add brine till the oil rises into the neck of the bottle and again centrifugalize for 10 min If the separation is not satisfactory or the liquid is not clear, cool to about 10° and centrifugalize for an additional 10 min Multiply the reading by 2 to obtain the percentage of oil by volume

OILS OF LEMON ORANGE, AND LIMES IN VEGETABLE AND MINERAL OILS

I By Steam Distillation[17]*—Official*

65 APPARATUS

(a) *Steam generator filled with H_2O* —An oil can holding 1 gallon will serve the purpose

(b) *Distillation flask* —A Kjeldahl flask of about 750 cc capacity, with shortened neck, about 10 inches in height over all

(c) *Spray tube* —A glass tube with a small perforated bulb at the end passes thru a rubber stopper and reaches to the bottom of the distillation flask

(d) *Bent glass tube* —About 8 mm in diameter Connects distillation flask to upright condenser The shape of this tube allows the vapor condensing in the tube to return to the distillation flask

(e) *Liebig condenser* —With 20-inch water jacket

(f) *Wilson receiving flask* —Shaped like a Babcock test bottle with a graduated neck but of much larger capacity and with a vertical glass outlet tube sealed on near the bottom The upper end of the outlet tube is turned down The capacity of the flask is about 250 cc The neck may consist of a portion of a buret graduated from 0–25 cc with top flared out The outlet tube is about 3 mm in diameter, and the end is at such a height that when the flask is filled with H_2O the meniscus in the neck will be between the 0 and 1 cc marks

66 DETERMINATION

Measure 100 cc of the sample in a graduated cylinder and transfer to the distillation flask Immerse the flask in a water bath and connect with the condenser by means of the bent glass tube Fill the receiving flask with H_2O and place under the condenser in such a way that the end of the condenser will be about 0 5 inch above the level of the H_2O in the receiving flask Place a 200 cc graduated cylinder under the end of the outlet tube to catch the displaced liquid Heat the water bath to boiling and pass steam thru the sample until 200 cc of liquid has been collected in the graduated cylinder

Disconnect the apparatus, allow the receiving flask to stand for 15 min or until separation of oil is complete, and read the volume of oil obtained Calculate the percentage (by volume) of essential oil in the sample by dividing the reading by 0 90 for lemon oil in corn and cottonseed oils, 0 95 for orange oil in corn and cottonseed oils, and by 0 78 for distilled or expressed oil of limes in corn and cottonseed oils Where the menstruum is mineral oil subtract 0 3 cc from the reading before dividing by the factors 0 90, 0 95 and 0 78 for lemon oil, orange oil and oil of limes respectively

67

II By Polarizations—Tentative

Polarize the sample at 20 in a 200 mm tube, making 5 readings From the average of these readings in degrees Ventzke subtract, for corn oil +0 6 for cottonseed oil −0 3°, for peanut oil +0 2 and for mineral oil +5 5°, as a correction for the rotatory effect of the menstruum To obtain the percentage by volume of the essential oil in the mixture, divide the corrected polariscopic reading so obtained by the factor 3 4 for lemon oil in corn oil 3 7 for lemon oil in cottonseed oil, 3 6 for lemon oil in peanut oil, 3 5 for lemon oil in mineral oil, 5 4 for orange oil in corn oil, 5 7 for orange oil in cottonseed oil, 5 6 for orange oil in mineral oil, 2 0 for oil of limes in corn oil, 2 3 for oil of limes in cottonseed oil and 2 2 for oil of limes in mineral oil

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XXVI FRUITS AND FRUIT PRODUCTS

1 PREPARATION OF SAMPLE—OFFICIAL

Transfer all samples received in open packages (i e , not in sterile condition) without delay to glass stoppered containers and keep in a cool place Make the determinations of alcohol, total and volatile acids, solids, and sugars, particularly in the case of fruit juices and fresh fruits, at once, as fermentation is liable to begin very soon (Portions for the determination of sucrose and reducing sugars may be weighed and kept for several days without fermenting if the slight excess of neutral Pb acetate soln required in the determination is added) The various products are prepared for analysis as follows

(a) *Juices* —Mix thoroly by shaking to insure uniformity in sampling and filter thru muslin previously washed and dried Prepare fresh juices by pressing the well pulped fruit in a jelly bag and filtering thru muslin previously washed and dried Express the juice of citrus fruit by means of one of the common devices for squeezing oranges or lemons and strain the expressed juice thru muslin previously washed and dried

(b) *Jellies and sirups* —Mix thoroly to insure uniformity in sampling

(b_1) *Preparation of soln* —Weigh 300 g of the thoroly mixed sample into a 2 liter flask and dissolve in H_2O, heating on a steam bath, if necessary Apply as little heat as possible to minimize inversion of the sucrose Cool, dilute to the mark, mix thoroly by shaking, and use aliquots for the various determinations If insoluble material is present, mix thoroly and filter before taking the aliquots

(c) *Fresh fruits dried fruits, canned fruits, preserves, jams, and marmalades* — Pulp by grinding in a large mortar or by passing thru a food chopper and mix thoroly completing the operation as quickly as possible to avoid loss of moisture In the case of dried fruits, pass the sample thru the food chopper three times, mixing thoroly after each grinding In the case of stone fruits remove the pits, and determine their proportion in a weighed sample In the case of canned fruits, an examination of the sirup in which the fruits are preserved is often sufficient Separate the liquor by draining (cf XXXV, 2) and treat as directed under (a)

(c_1) *Preparation of soln* —Weigh 300 g of the well pulped and mixed sample into a 1 5–2 liter beaker, add about 800 cc of H_2O, and boil 1 hour, replacing at intervals the H_2O lost by evaporation Transfer to a 2 liter volumetric flask, cool, dilute to volume, and filter With unsweetened fruit it is desirable, tho not actually necessary, that sugar be added before boiling, therefore weigh 150 g of fruit add 150 g of sugar and 800 cc of H O and proceed as directed above

2 ALCOHOL—OFFICIAL

Determine alcohol in 50 g of the original material as directed under XXXIV, 79

3 MOISTURE

DRIED FRUITS (GENERAL)—OFFICAL

Spread 5–10 g of the sample, prepared as directed under 1 (c), as evenly as possible over the bottom of a metal dish approximately 8 5 cm in diameter and provided with a tightly fitted cover, weigh, and dry at 70° for 12 hours under a pressure not to exceed 100 mm of Hg During the drying admit to the oven a slow current of air, about 2 bubbles per second, dried by passing thru concentrated H_2SO_4 (The metal dish must be placed in direct contact with the metal shelf of the oven) Replace the

cover, cool in a desiccator, and weigh Disregard any temporary drop of oven temp that may occur during the early part of the drying period owing to rapid evapora tion of H_2O With raisins and fruit similarly rich in sugar use about 5 g of sample and dry and weigh with the dish about 2 g of finely divided asbestos Moisten with hot H_2O, mix the sample and asbestos thoroly, evaporate on a steam bath barely to dryness, and complete drying as directed above

DRIED APPLES—TENTATIVE

4 (A rapid method applicable only to dried apples)

Spread 5–10 g of the sample prepared as directed under 1 (c), as evenly as pos sible over the bottom of a metal dish approximately 8 5 cm in diameter and pro vided with a tightly fitted cover, weigh, and dry for 4 hours in an oven at the temp of boiling H_2O Replace the cover cool in a desiccator, and weigh Place the dish on one of the shelves—not on the oven bottom Provide a vent in the top of the oven to insure ventilation

TOTAL SOLIDS

FRESH AND CANNED FRUITS JAMS MARMALADES AND PRESERVES—OFFICIAL

5 (Insoluble matter present)

Weigh accurately 20 g of pulped fresh fruit or a quantity of fruit products that will give not more than 3–4 g of dry material into a large flat bottomed dish If necessary to secure a thin layer of the material add a few cc of H O and mix thoroly Dry at 70 under a pressure of not to exceed 100 mm of Hg until consecutive weigh ings made at intervals of 2 hours do not vary more than 3 mg

FRUIT JUICES JELLIES AND SIRUPS—OFFICAL

6 (No insoluble matter present)

Proceed as directed under XXXIV, 3, 5, 6 or 7, using the sample prepared as di rected under 1 (a) or (b)

WATER INSOLUBLE SOLIDS—TENTATIVE

7 Weigh 25 g of the sample prepared as directed under 1 (c) into a 400 cc beaker, add 200 cc of H_2O, cover heat to boiling and boil vigorously for 30 min replacing at intervals the H_2O lost by evaporation Prepare about 0 5 g of absorbent cotton or a coarse qualitative 15 cm filter paper by drying in a water oven and weighing in a flat bottomed dish provided with a cover Filter the soln thru the absorbent cotton or filter paper and wash the residue and filter with hot H O until the wash H O is colorless and no longer acid to litmus paper Transfer the cotton or paper filter con taining the insoluble solids to the flat bottomed dish, dry to constant weight in a water oven, and weigh The increased weight represents the water insoluble solids in the sample taken The filter of absorbent cotton may be prepared by forming it into the shape of the funnel and by means of a wire or glass rod forcing a pledget of the cotton into the stem, but leaving the portion against the sides loose

TOTAL ASH—OFFICIAL

8 Proceed as directed under XXVII, 8, the temp of ashing not to exceed 525° using 2 g of juices fresh fruits, and canned fruits and 10 g of jellies, sirups preserves jams, marmalades and dried fruits

If the ash of the water soluble portion only is desired, evaporate to dryness 100 cc of the soln prepared as directed under either 1 (b_1) or 1 (c_1) and proceed as directed under XXVII, 8

9 ALKALINITY OF THE ASH—OFFICIAL

Into the Pt dish containing the ash obtained under 8 introduce a measured excess of 0 1 *N* HCl, warm on a steam bath, cool, add a few drops of methyl orange indicator, and titrate the excess acid with 0 1 *N* NaOH soln Report the result as alkalinity, the number of cc of 0 1 *N* acid required to neutralize the ash from 100 g of sample, and as alkalinity number, the number of cc of *N* acid required to neutralize 1 g of ash Reserve the filtrate for the determination of S in ash

10 SULFUR IN ASH—OFFICIAL

(For products containing a basic ash)

Add 5 cc of HCl (1+2 5) to the soln remaining after the determination of alkalinity of ash under 9 and evaporate to dryness Heat to 110° for 1 hour to dehydrate any SiO_2 Take up in 5 cc of the dilute HCl and filter, washing the filter paper well with hot H_2O Heat the filtrate to boiling and add dropwise from a buret or pipet 5 cc of 10% $BaCl_2$ soln Evaporate to 100 cc and let stand overnight Filter on a weighed Gooch or Munroe crucible or on a 7 cm ashless filter paper, wash with hot H O until the filtrate is free from chlorides, dry, ignite over a Bunsen burner, and weigh as $BaSO_4$ As the quantity of precipitate is small exercise great care and make the determination in duplicate Report the result as mg of S per 100 g and as percentage of S in ash

11 TOTAL SULFUR—TENTATIVE

(For sulfured products and for samples containing little ash or an acidic ash)

In a casserole as large as can be placed in the electric muffle furnace availa— place 1–3 g of MgO (1 g for fruit juices, 3 g for heavily sugared products and or dried fruits—or an equivalent quantity of $Mg(NO_3)_2$), 1 g of powdered sucrose the 50 cc of HNO_3 Then add 5–10 g of the sample prepared as directed under 1 (a) hor- or (c) Place the same quantities of the reagents in another casserole for a bmine Evaporate on a steam bath to a pasty consistency Place the casserole in a col ation tric muffle and gradually heat to not above dull redness until all N_2O_4 fume liquor been driven off (All organic matter will have been destroyed) Cool, dissolve (1+2 5), and filter Adjust the acidity so that the soln contains 0 5–1 g of fr ple into heat to boiling, and add dropwise 5 cc of a 10% $BaCl_2$ soln Evaporate to tervals allow to stand overnight, filter, wash, ignite, and weigh the $BaSO_4$ Correc ilute to sult for the $BaSO_4$ obtained in the blank and report as mg of S per 100 g Tr ces termination should be made in a room free from S fumes of

CHLORINE IN ASH

12 *Method I—Official**

13 *Method II—Tentative*

Proceed as directed under XII, 33 and 35

14 POTASSIUM—TENTATIVE

REAGENTS

(a) *Ammonium chloride soln* —Dissolve 100 g of NH_4Cl in 500 cc of H O, add 5–10 g of pulverized K_2PtCl_6 and shake at intervals for 6–8 hours Allow the mix

* See note 7 p xvii

ture to settle overnight and filter (The residue may be used for the preparation of a fresh supply)

(b) *Platinum soln*—For materials containing less than 15% of K_2O a $PtCl_4$ soln containing 0 2 g of metallic Pt (0 42 g of H_2PtCl_6) in each 10 cc is recommended

(c) *Alcohol*—90% Sp gr 0 8339 at 15 6/15 6°

PREPARATION OF SOLUTION

15 (A) Dissolve the ash in HCl If it is desired to take an aliquot filter in a volu metric flask, wash the filter thoroly, and make up to volume Pipe an aliquot into a beaker, adjust to a volume of 50–75 cc, heat to boiling and add a slight excess of NH_4OH and then sufficient saturated NH_4 oxalate soln to precipitate all the lime and Al present Filter into a large Pt dish and wash the filter thoroly

DETERMINATION

16 Evaporate the soln from (A) nearly to dryness add 1 cc of H_2SO (1+1), evapo rate to dryness, and ignite to whiteness Maintain a full red heat until the residue is perfectly white Dissolve the residue in hot H_2O, using at least 20 cc for each dg of K_2O present, add a few drops of HCl and then an excess of Reagent (b) Evapo rate on a water bath to a thick paste avoiding exposure to NH_3 Treat the residue with 60% alcohol Filter on a dry tared Gooch crucible with an asbestos mat that has been washed thoroly with 90% alcohol and dried at 100 for 30 min Wash the pre cipitate thoroly with 90% alcohol, both by decantation and on the crucible mat, and continue the washings after the filtrate is colorless, using about 200 cc of wash solns Then wash 5 or 6 times with 10 cc portions of the NH_4Cl soln to remove im purities from the precipitate Wash again with four or five 10 cc portions of 90% alcohol and dry the precipitate for 30 min at 100° Weigh, wash again with several cc portions of 90% alcohol dry, and reweigh until a constant weight of K_2PtCl_6 is obtained Calculate to K_2O The precipitate should be completely soluble in H_2O

MANGANESE—TENTATIVE

REAGENTS

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(a) *Sodium hydroxide soln*—10% freshly prepared

(b) *Sodium oxalate soln*—Saturated

(c) *Oxalic acid soln*—3%

(d) *Sodium acetate soln*—20%

(e) *Sodium dihydrogen phosphate*—10%

(f) *Ammonium hydroxide soln*—100 cc of NH_4OH diluted to 1 liter

(g) *Hydrochloric acid*—5 cc of HCl diluted to 30 cc

(h) *Ammonium oxalate soln*—Saturated

PREPARATION OF SOLUTION

18 (B) Dissolve the ash in HCl, evaporate to dryness and bake at 110 for 1 hour to dehydrate any SiO_2 Dissolve the residue in the dilute HCl and filter into a volu metric flask Wash the filter thoroly and make up to volume

DETERMINATION

19 To an aliquot of soln B add sufficient Br water to oxidize any ferrous Fe to the ferric state Boil off the excess Br Dilute to 150 cc and heat to boiling Add sufficient NaH_2PO_4 (e) to combine with all the Fe and Al present Add plenty of bromecresol green indicator and while the mixture is gently boiling add NaOH (a) dropwise to